UNIVERSITY
OF TASMANIA

QUANTIFYING AND PREDICTING BENTHIC ENRICHMENT: LESSONS LEARNT FROM SOUTHERN TEMPERATE AQUACULTURE SYSTEMS

By

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STATEMENTS AND DECLARATIONS

1.1 Declaration of Originality

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Nigel Brian Keeley

1.4 Statement of Co-authorship

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Located in chapter 4

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Paper 6, Benthic recovery and re-impact responses from salmon farm enrichment: implications for farm management

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Several of the research papers draw upon a long-term environmental dataset that has been contributed to by many people over the last 20 years. Most notably, the following people have either been responsible for instigating earlier studies, or collecting information: Dr Steven Brown (NIWA, Nelson), Dr Barrie Forrest, Dr Paul Gillespie, Murray Clark, Dr Grant Hopkins, Reid Forrest, Dr Robyn Dunmore and Dr David Taylor (all of Cawthron Institute). I am grateful for their assistance and contributions. The extensive experience of several of these people was also utilised in the 'assessment of best professional judgement' process, as acknowledged in Chapter 2. I would like to say a special thanks to Dr Paul Gillespie, although not directly involved in the preparation of these research papers, has provide invaluable wisdom, experience and a balanced view over the last several years; which I have frequently drawn upon.

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Finally, I want to say a very big thank you to my partner Ellie, as well as to family and friends / colleagues, for their support and encouragement during what is, in essence, a fairly selfish period of one's life. I hope to be able to repay your patience and support in the near future.

GENERAL ABSTRACT

The increasing demand for aquaculture products globally is leading to greater demand for coastal marine farm space, intensification within existing aquaculture areas, and conversion of production to high value species, especially finfish. Among the many environmental interactions that arise with finfish aquaculture development, one of the most dramatic impacts is local-scale organic enrichment of the benthic ecosystem due to deposition of fish faeces and uneaten feed. A benthic impact is typically evident as severe organic enrichment beneath finfish cages (e.g. species-poor, near-azoic conditions), with a gradient of decreasing enrichment extending to background conditions across scales of tens to hundreds of metres distant from cages.

The overall hypothesis of this thesis was that seabed organic enrichment (degradation and recovery) can be accurately and quantitatively determined using biological and physico-chemical variables that can be applied across geographic regions and contrasting environments. This was accompanied by an objective to refine knowledge of processes underpinning benthic enrichment, and to develop or refine tools for the prediction, monitoring and management of enrichment effects associated with fish aquaculture. The thesis comprises six sequential, related chapters that address: site- and region-specific ecological characterisation of benthic communities and the development on a new environmental indicator variable; comparisons of existing biological indicators and indices in different hydrodynamic regimes; application and validation of a depositional model for predicting effects under very different environmental conditions; and a detailed analysis of long-term and medium-term recovery from highly enriched states, and consideration of re-impact rates and implications for farm management strategies. The analyses are based on both targeted recent studies as well as longer-term monitoring undertaken at six salmon farms situated in the Marlborough Sounds, New Zealand; four of which are situated in low flow environments, and two are situated in high flow (dispersive) environments. Characterising the differences associated with the sites' dispersive properties is a theme that runs throughout this study.

Chapter 2 used best professional judgement methods to develop a quantitative benthic enrichment index termed 'enrichment stage', which unifies information from biological and

physico-chemical variables. The resulting seven stage bounded continuous variable was used to assign enrichment tolerance groups to benthic taxa using quantile regression splines. A number of key indicator taxa were discriminated along the enrichment gradient, including several that were responsive to low-level changes in enrichment stage (ES), but not necessarily organic matter (%OM), and 10 taxa for which ecological understanding was previously limited. In Chapter 3, the gradient was also used to evaluate the performance of five benthic indicators and ten biotic indices for defining organic enrichment under different flow regimes. A subset of variables was recommended comprising: two biotic indices, total abundance, and a geochemical variable. A subsequent but related study in Chapter 4 revealed pronounced flow-related differences in the magnitude and spatial extent of benthic enrichment. Total macrofaunal abundances at high-flow sites were nearly an order of magnitude greater than at comparable low flow sites, representing a significant benthic biomass, and occurred in conjunction with moderate-to-high species richness and the absence of appreciable organic accumulation. The atypical ecological conditions associated with high-flow sites were attributed to i) minimal accumulation of fine sediments, ii) maintenance of aerobic conditions in near-surface sediments, and iii) an abundant food supply.

Chapter 5 explored the relationship between predicted depositional flux (using DEPOMOD) and enrichment stage, calculated using the methods developed in the previous chapters (1 to 3). Observed impacts at farms with contrasting flow regimes were examined to evaluate the role of modelled resuspension dynamics in determining impacts. When resuspension was included in the model, net particle export was predicted at the most dispersive sites. However, significant seabed effects were observed, suggesting that although the model outputs were theoretically plausible they were inconsistent with the observational data. When the model was run without resuspension the results were consistent with the field survey data. This retrospective validation suggested that approximately twice the flux was needed to induce an effect level at the dispersive sites equivalent to that at the non-dispersive sites. Flux estimates are provided for detectable enrichment and highly enriched states. This study shows that the association between current flow, sediment resuspension and ecological impacts is more complex than presently encapsulated within DEPOMOD and emphasises the need for validation of such models, particularly at dispersive sites.

The final two data chapters (Chapters 6 and 7) examine the spatial and temporal recovery processes that take place following a highly enriched state. Chapter 6 provides a

comprehensive analysis of a long-term (8 year) dataset in relation to a variety of proposed recovery and remediation definitions. Many challenges associated with quantifying the endpoint of 'recovery' were identified. The concept of dynamic and spatial equilibria proved to be valid in this situation, and alternate state theories may apply. In combination with visualisation of plotted data, statistical tests for parallelism in temporal trajectories of cage and reference sites proved to be an effective method for characterising recovery, but the method was highly sensitive to window time-length. Simple, univariate indicators of enrichment tended to be less sensitive, and indicate recovery earlier, than more complex multivariate indicators. Recovery was assessed to be complete after approximately five years, but there was some evidence of on-going instability in the composition of the macrofauna, which was partly attributed to spatial and temporal processes and patterning in the macrobenthos. The last data chapter (Chapter 7) examined shorter-term recovery and re-impact patterns and revealed some interesting successional patterns in time and space, especially between %OM, TFS and abundances of opportunistic taxa. The discussion brings together findings from the targeted and long-term studies to reveal alternate oscillations between sediment chemistry and biological response, which have temporally distinct signals. It is proposed that the large oscillations that occur in the early stages of recovery represent the extreme end of the environmental instability that occurs as a result of a severe perturbation (in this case, cessation of extreme enrichment) that abates through time as recovery ensues.

This integrated study has a number of important implications for the management of organic enrichment in general but is especially pertinent for fish farming. In particular, recommendations are made regarding the i) adequacy of chemical and biological benthic indicators and their performance in typical non-dispersive and atypical dispersive sites; ii) use and applicability of depositional models in the same environments with emphasis on the role of resuspension, and iii) timing and approach for reintroduction of impacts, with respect to monitoring and management of rotational fallowing strategies to ensure on-going sustainability.

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CHAPTER 1

GENERAL INTRODUCTION, OVERVIEW AND THESIS STRUCTURE

1.1 Background

Human-induced pollution of aquatic ecosystems is a major global environmental issue. The primary human-mediated pathways that lead to polluted water-bodies include agriculture and horticultural discharges of fertilizers and stock effluent (Howarth et al. 2002, Smith et al. 2006), and point-source inputs of human and industrial wastes (Taylor et al. 1998, Bothner et al. 2002). The primary receiving environment is often the freshwater system (Foley et al. 2005); however, the ultimate receiving environment is invariably the ocean, where catchment-derived inputs and direct discharges can strongly influence the physical, chemical and biological properties of the near-shore coastal environment (Pagola-Carte & Saiz-Salinas 2001, Galope-Bacaltos & San Diego-McGlone 2002, Steckbauer et al. 2011). In addition to these various land-derived sources of pollution, additional threats to the coastal environment arise from processes that are linked to human perturbations on a global scale (e.g. ocean acidification), direct discharges from ocean outfalls (Taylor et al. 1998, Bothner et al. 2002), as well as human activities that occur within the marine environment, such as seabed mining, fishing and aquaculture (Jones 1992, Jennings et al. 2001, Brooks et al. 2002, Bolam et al. 2005, Borja et al. 2006, Somerfield et al. 2006). Collectively, these human-induced forms of disturbance have the potential to result in large-scale impacts (e.g. coastal hypoxia or 'dead zones') that represent a major threat to coastal ecosystems and associated biota (Steckbauer et al. 2011). Protecting the integrity of aquatic ecosystems is therefore of great importance and managing the effects is a global challenge (Foley et al. 2005, http://www.unesco.org/new/en/natural-sciences/ioc-oceans/high-level-objectives/ecosystem-health/#c110213).

The need to effectively manage and mitigate effects from marine pollution is also rapidly increasing in accordance with an expanding human population and the associated production of wastes and demands for natural resources. Demand for sea-based food products exemplifies this problem; there is an increasing demand for seafood driven by population growth, which is compounded by increasing awareness of the benefits of eating seafood and improved socio-economic conditions in developing countries (Jensen et al. 2001, Corbin 2007, Dey et al. 2008, Lindkvist et al. 2008, Pitcher 2008). This increasing demand is occurring in the face of static or globally declining fish stocks (Pauly et al. 2002, Pauly 2004, Jiang 2009) and as a result there is mounting pressure on aquaculture to bridge the gap between supply and demand. World aquaculture production reached approximately 62.7 million tonnes, and had a net worth of US$130 billion, in 2012 after three decades of expansion, and further growth is anticipated (FAO 2013). The increasing demand for aquaculture products will ultimately be met by expansion into new areas (e.g. offshore) and modification of existing operations; i.e. allocation of new areas for farming, intensification within existing allocated areas and, in some cases, conversion of production to higher value species such as finfish.

The situation in New Zealand reflects the global scene; the aquaculture industry has significant expansion targets in place to be met by 2025[1], and the Environmental Protection Agency recently convened a Board of Inquiry to consider a large application for increased salmon farming space[2]. Likewise, in Australia, major aquaculture expansions are planned, especially in Tasmania[3], where a 362 ha fish farm expansion in Macquarie Harbour has recently been approved[4]. However, aquaculture production, and the environment that sustains it, are both vulnerable to adverse impacts from degrading conditions (FAO 2012), with the potential for significant negative environmental consequences to arise as production exceeds the carrying capacity of the environment (Inglis et al. 2000, Stigebrandt et al. 2004, Gyllenhammar & Hakanson 2005; Forrest et al. 2007; Keeley et al. 2009, Buschmann et al. 2006, Kalantzi & Karakassis 2006). As a result, and because of uncertainty with respect to the magnitude and significance of the adverse effects of aquaculture development, consent applications to expand aquaculture operations have proven highly controversial and can polarize

[1] http://aquaculture.org.nz/industry/overview/
[2] http://www.epa.govt.nz/Resource-management/King-Salmon/Pages/default.aspx
[3] http://www.dpiw.tas.gov.au/inter.nsf/WebPages/ALIR-4YS3VE?open
[4] http://www.salmonfarming.org/news/tasmanian-atlantic-salmon-industry-expansion-approved/

communities[5]. There is clearly a need to better understand and predict the likely impacts of existing activities and of proposed expansions, in order to ensure the natural virtues of the regions are not compromised and are sustainable (Gibbs 2009).

1.2 Impacts of aquaculture

The need to understand the effects of aquaculture on the environment tends to be even more important in New Zealand and other developed countries where marine farms are often situated in areas of relatively high ecological quality, in part because the culture organisms themselves (e.g. fish, shellfish) intrinsically demand high levels of water quality. For example, salmon require cool, clear, well oxygenated water with a narrow pH band to sustain good growth and health (Groot & Margolis 1991, Staurnes et al. 1995), and shellfish growth can be impaired by toxic algae blooms (Chauvaud et al. 1998) which are often linked to river plumes and other nutrient rich discharges (Anderson et al. 2008). High water quality is also necessitated by the food and health regulations that are associated with growing food for human consumption (e.g. Australia New Zealand Food Standards Code, ANZFSC 2002). Poor environmental conditions can prevent harvesting and sales of product if contaminants or toxic compounds are present (e.g. toxic algae in Greenshell mussels, James et al. 2010).

Due to increased recognition of the importance of understanding aquaculture effects, a number of recent studies have provided a synthesis of environmental interactions (Forrest et al. 2007, Forrest et al. 2009, MPI 2013). In terms of broad categories of impact, recognised environmental interactions (see Figure 1-1) can include wider ecosystem effects that arise as a result of factors such as nutrient release and impacts on water column production (e.g. elevated nutrients leading to algal blooms: Brooks et al. 2002, Buschmann et al. 2007), pest or disease transmission (Forrest et al. 2007), interactions with wild fish populations (Dempster 2005, Dempster et al. 2006), and with wildlife such as seabirds (e.g. Roycroft et al. 2004, Kirk et al. 2007) and marine mammals (Kemper et al. 2003, Markowitz et al. 2004). Whereas many of these broader interactions can be difficult to quantify (but may nonetheless be important), it is at a more localised scale that impacts are most pronounced and more readily quantifiable. In particular, changes to the seabed environment in the vicinity of culture areas tend to be one of

[5] http://www.stuff.co.nz/marlborough-express/news/7539693/Salmon-farms-reel-in-conflict, https://www.et.org.au/world-heritage-under-threat-and-no-real-science-available

the most obvious and measurable effects of aquaculture on natural ecosystems (e.g. Hargrave et al. 1997, Janowicz & Ross 2001, Karakassis et al. 2002, Thetmeyer et al. 2003, Buschmann et al. 2006). Benthic impacts can provide a time-integrated picture of recent events, and as such lend themselves to routine monitoring for environmental compliance purposes (e.g. Wilson et al. 2009).

Benthic effects arise in a range of ways, and can differ for different types of aquaculture (MPI 2013); however, it is widely recognised that the most significant benthic impacts in coastal environments arise as a result of sea-cage (also called net pen) fish farming (e.g. Karakassis et al. 2000, Crawford et al. 2003, Edgar et al. 2005), or from comparable forms of aquaculture (e.g. shrimp farming) where substantial quantities of protein rich feed are added on a daily basis. For example, a salmon farm that produces 1000 tonnes of fish annually will use approximately 3 tonnes of feed per day (assuming an FCR of 1.1, Buschmann et al. 2007). Up to 10 % of the feed remains uneaten and falls to the seabed (Chamberlain & Stucchi 2007, Cairney & Morrisey 2011), and approximately 13 % of the feed is processed by fish and re-enters the water column as faecal particles, which also gets deposited on the seabed in the immediate vicinity of the farm (Cromey et al. 2002a). Collectively these organic-rich particles are referred to as biodeposits. Although trace contaminants can be present in biodeposits (e.g. copper and zinc, Brooks & Mahnken 2003b), the most well-recognised changes to the benthos in the vicinity of feed-added aquaculture installations are associated with organic enrichment of the seabed.

The focus of this thesis is the increased understanding and prediction of the spatial and temporal responses of the benthic environment to enrichment, and the associated development of indicators and evaluation of mitigation techniques. Fish farms provide a particularly useful model system for studying organic enrichment effects, and for testing potential environmental monitoring and assessment tools, for a number of reasons. First, the magnitude of benthic impacts can be extreme, and typically more pronounced than most other coastal activities where organic enrichment occurs (e.g. raw or treated sewage discharges; Roper et al. 1989, Taylor et al. 1998). These extreme impacts can abate to background levels over spatial scales of tens to hundreds of metres, providing a strong gradient of impact for scientific investigation (Brown et al. 1987, Karakassis et al. 2000, Brooks & Mahnken 2003a). It is also relevant that environmental changes along the gradient can be readily linked to fish farm activities, as the isolated nature of aquaculture sites tends to

geographically isolate them from the confounding effects of other human activities. Finally, the expansion of aquaculture means that multiple fish farm sites occur at a regional scale across contrasting environments, which enables enrichment effects and management tools to be evaluated in a robust manner.

1.3 Benthic enrichment from sea-cage fish farms and state of current knowledge

1.3.1 The enrichment process

Benthic enrichment refers to the physico-chemical processes and ecological responses that occur as a result of deposition to the seabed of the organic-rich farm-derived biodeposits. As the excess volatile organic matter degrades, oxygen demand increases, and the underlying sediments become deoxygenated, with sulphate reducing bacteria converting sulphate to sulphides (Hargrave et al. 2008). These processes result in a lowered redox (oxidation-reduction) potential and increased sediment sulphide concentrations. The sulphides produced are toxic to most animals, which display a range of tolerances (Hargrave et al. 2008). Once the sulphate is depleted, methanogenesis becomes the dominant metabolic process, often producing methane gas. As a result, benthic communities can become highly modified and 'enriched', infaunal diversity will be significantly reduced and extreme abundances of opportunistic, sulphide-tolerant taxa may occur. In extreme cases sediments can become anoxic (without oxygen) resulting in zones that can be azoic, meaning that they are devoid of life other than certain micro-organisms (e.g. the bacterium *Beggiatoa* spp.) that thrive in such environments (Gowen & Bradbury 1987).

1.3.2 Limitations of present approaches to measuring and predicting benthic enrichment

There remain some significant knowledge gaps that impact our ability to reliably predict and assess benthic effects of finfish aquaculture, which are the focus of this thesis. One of the main short-comings of benthic monitoring of fish farms is that the choice of indicators has been inconsistent between individual assessments, organisations, regions and countries (e.g. Wilson et al. 2009). In some cases qualitative and subjective parameters have been used, or quantitative physico-chemicals metrics are adopted as simple monitoring indicators without

necessarily correlating well with ecological responses in different farm environments. There are numerous existing biotic indices (Pinto et al. 2009) that have been established for assessing benthic impacts for other purposes (Pinto et al. 2009), however, there uptake for aquaculture purposes has been slow. Most of these utilise the established successional response (Pearson & Rosenberg 1978, Gray et al. 1979) that is strongly expressed in full beneath fish farms, and therefore have potential for discerning those effects (Borja et al. 2009b). There are also aquaculture regions that tend to place more emphasis on geochemical, rather than biological, variables as the primary indicators of effects (Wildish et al. 2004, Hargrave et al. 2008). As a result, the ability to make comparisons with, and learn from, the experiences of other regions or countries has been impaired. Simultaneously, there remains a need for improved enrichment indicators that integrate physico-chemical and ecological responses, and have general applicability across the range of environments in which fish farms are situated. Hence for this thesis, there exists a valuable basis for comparison and evaluation of potential benthic environmental indicators and methods, as well as for their further development and extension for use with predictive tools.

The size, shape and intensity of the "footprint" of benthic effects is often used as a basis for managing in relation to acceptable zones of effects, or AZE (e.g. Wilson et al. 2009, Figure 1-2, ASC 2012). The footprint is strongly influenced by the bathymetry and hydrodynamic properties of the farm site, as strong currents will promote waste dispersion (Cromey et al. 2002b) and oxygen delivery to the sediments (Findlay & Watling 1997). Strongly-flushed or "high flow" sites are generally thought to be more resilient to benthic enrichment (Frid & Mercer 1989, Borja et al. 2009b); however, the relationships between current speed, deposition flux rates and the benthic response remain poorly defined. Accordingly, predictive approaches to environmental management (i.e. modelling) are still largely in the developmental stage and vary greatly in terms of methods employed, ease of application effectiveness and importantly, reliability. These are important considerations that are addressed in this thesis, as both the spatial extent and magnitude of effects are key decision criteria for assessment of overall impact with respect to resource management (see below).

Also critical to any assessment of effects, and of approaches to mitigation, is the concept of benthic recovery, as it concerns the longevity and reversibility of any effects, should the farm be removed. To date "recovery" has been defined in different ways, with no consensus on appropriate assessment methods. Estimates of putative "recovery" vary greatly, from less

than six months (Ritz et al. 1989, Brooks et al. 2003) to more than 5 years (Brooks et al. 2004), with many studies not being of sufficient duration to identify "complete" recovery; i.e. to pre-impact condition or to conditions consistent with those of selected control sites (e.g. Karakassis et al. 1999, Macleod et al. 2004c). An understanding of recovery rate is critical from a farm management perspective, as sites are regularly fallowed (temporarily destocked) to allow the sediments to return to a less enriched and impacted state. The relevance and sustainability of fallowing as a management practice from a benthic perspective remains a contentious issue (Brooks et al. 2003, Hall-Spencer & Bamber 2007) because it requires more space and impacts a greater area of seabed (i.e. because the stock from the fallowed site is moved to a new site). Relative rates of recovery versus rates of impact (or re-impact) at new sites have important implications for the overall farm space required and the sustainability of fallowing cycles, but have not yet been considered in any detail.

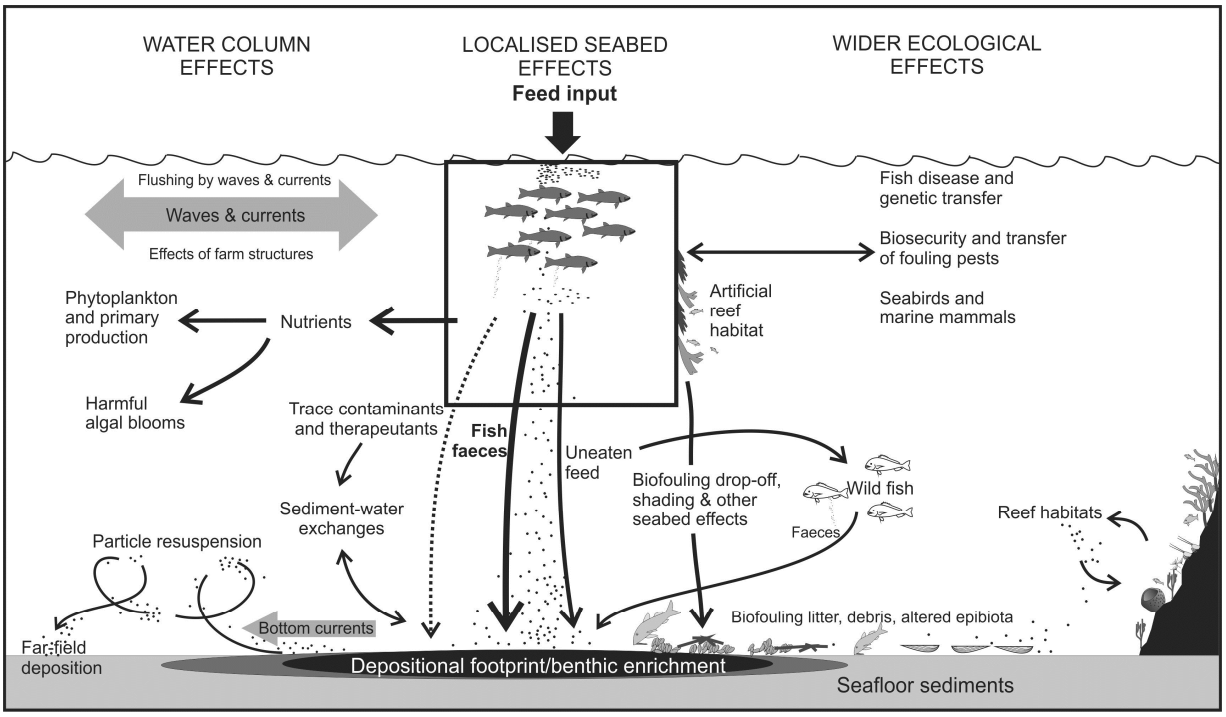

Figure 1-1: Stylistic summary diagram of the potential types of ecological effects associated with enrichment from salmon farm aquaculture.

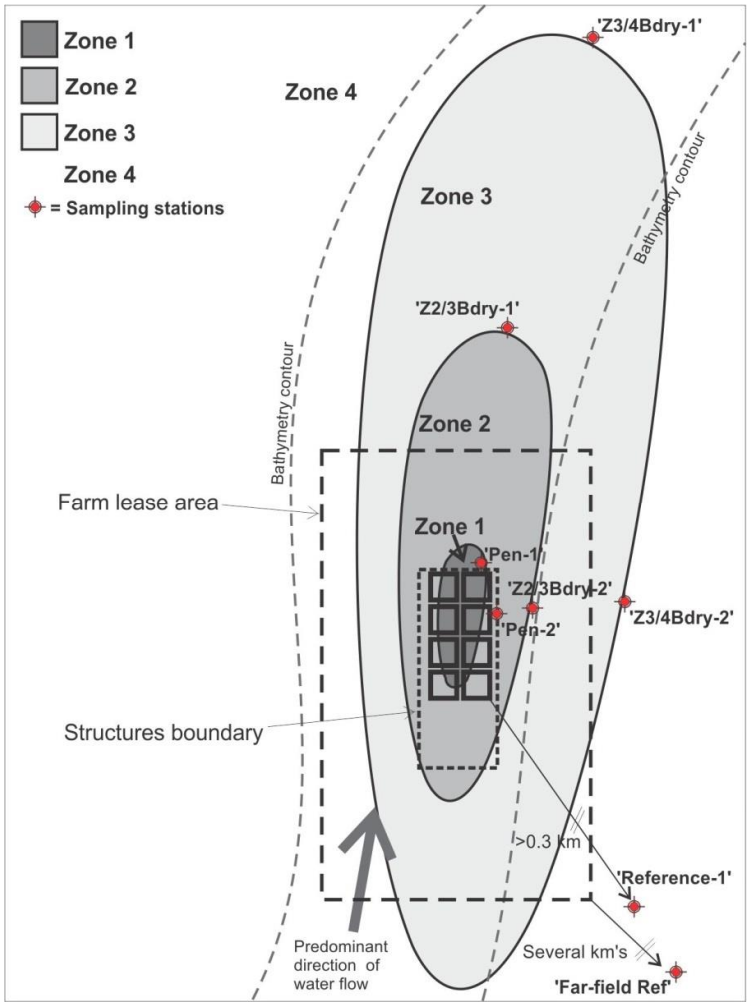

Figure 1-2: Generic representation of a 'benthic footprint' beneath a salmon farm indicating potential deformation in response to flow and arrangement of 'impact zones' and sampling stations for monitoring and management purposes in New Zealand. At most farms, the footprint or AZE is constrained by three zones each with an associated tolerable level of effect and/or specified 'environmental quality standards' (EQS). Zone 1 - Directly beneath the cages; impacts can be quite severe although excessive enrichment resulting in anoxia and azoic conditions is prohibited. Zone 2 – allows for moderate to high levels of enrichment out to a distance of approximately 50 m at low/ moderate flow sites (greater for high flow sites). Zone 3 -effects are expected to be negligible (i.e. approximate natural conditions) at a set distance away from the farm cages (usually 150 m for low flow sites). Monitoring is generally undertaken at the boundaries for each of the zones (Figure 1-2).

1.4 Primary aims and structure of this thesis

The overall hypothesis of this thesis was that seabed organic enrichment (degradation and recovery) can be accurately and quantitatively determined using biological and physico-chemical variables that can be applied across geographic regions and differing environments. This hypothesis was accompanied by an objective to refine knowledge of processes underpinning benthic enrichment, and develop or refine tools for the prediction, monitoring and management of enrichment effects associated with fish aquaculture. This was based on a scientifically robust analysis of selected quantitative methods for environmental monitoring and management worldwide, and an assessment of the relative performance of these approaches under New Zealand conditions (e.g. a poorly described benthic ecology). A key component of this study was not just an assessment of environmental degradation processes associated with aquaculture operations, but also consideration of the recovery potential, such that the full cycle of environmental impacts under a broad range of different environmental conditions can be considered, and the most effective management strategies implemented. In order to achieve these aims, I derived a complex and extensive dataset, combining targeted short-term studies with a large long-term dataset covering 10 years of environmental monitoring beneath six established commercial salmon farms (representing 75 % of New Zealand's sea-cage salmon farms). This end result is a balanced prognostic/diagnostic management approach that has global applicability.

The overarching hypothesis has been addressed through a series of six sequential and related studies, presented as chapters, each with their own specific goals, but which together contribute to the broader purpose. The final chapter (Chapter 8, General Discussion) summarises the main findings and presents the key environmental management recommendations that arose from the work. The aims and general content of the individual chapters are as follows:

Chapter 2 presents a means of evaluating enrichment sensitivities for poorly described taxa, and for assigning new, or validating existing, enrichment tolerance classifications, to increase the relevance and application of existing biotic indices to new regions. This was necessary because although biotic indices have been developed and readily applied in the northern hemisphere, their application assumes a reasonable level of knowledge of, and agreement upon, endemic macrofauna in terms of their enrichment tolerance, which did not

exist in the study region. There is also evidence to suggest that the transferability of biotic indices and the underpinning enrichment tolerance classifications between regions may be problematic. To address this, I developed a novel means of unifying biological and physico-chemical variables to produce a single variable (Enrichment stage, ES) that represented the key stages of enrichment-related degradation. The development of this ES variable provided an alternative, quantitative method to the traditionally subjective approach to classifying new taxa.

The motivation for **Chapter 3** was to identify which variables, or suite of variables, best characterise enrichments effects across the full spectrum of enrichment, with emphasis on versatility and their ability to provide relevant impact classifications in different flow environments. The need for versatility with respect to different flow environments was considered important, as preliminary observations suggested that the way in which the benthic ecology responded to enrichment was different to that commonly described for "low flow" sites where most fish farming takes place globally, and where most studies to date have been conducted. Furthermore, there appears to be a present shift in the fish farm industry toward high flow sites, and the gap in understanding around how high flow sites respond to enrichment needed to be addressed. Using the ES variable, I was able to empirically describe the relationships between common infaunal and physico-chemical variables as they respond to enrichment. Identifying the most reliable indicator variables was an important step in being able to predict effects from depositional models that are examined in Chapter 5.

In **Chapter 4** I more closely examine the relationships among and between biological and physico-chemical indicators (e.g. total free sulphide), and compare species richness (S), abundance (N) biomass (B) and trends under different flow regimes against the responses that characterise a classic conceptual model for organic enrichment developed by Pearson & Rosenberg model (1979). I then reviewed these findings and their relevance to current understanding of successional responses, identifying the strengths and limitations of different environmental indicators for monitoring. Chapter 4 is therefore a logical extension of Chapters 2 and 3.

Chapter 5 utilises an existing model that was designed to predict depositional flux rates and organic accumulation beneath salmon farms in Scotland. The motivation to apply and further test this model at the study sites arose because: i) confident model application is

contingent upon regional validation, which is especially important where the hydrodynamic properties are relatively unique, and ii) the links between depositional flux rates and observed ecological effects are poorly described. So in this chapter I evaluate the strength of the link between model predictions and observed ecological responses by validating the model at the six study sites. I also develop empirical models to convert between predicted flux and observed effects for dispersive (high flow) and non-dispersive (low flow) sites, and in doing so provide a novel insight to scientific understanding of the role of seabed particle re-suspension.

Chapters 6 and **7** provide a detailed analysis of recovery and re-impact in the sediments beneath fish farms. Chapter 6 utilises a 10 year dataset detailing recovery from a highly impacted state at a low flow site. It also provides a good framework for evaluating different definitions and metrics of recovery, as well as some fundamental ecological concepts (e.g. the role of key taxa in remediation, and ecological succession end-points). Chapter 7 examines recovery rates more intensively over a shorter period (2 years) and contrasts these with re-impact rates at an adjacent site. These findings have important implications for fallowing and mitigation strategies that are often employed to manage seabed effects, and therefore the sustainability finfish farming in some locations.

The final **Chapter (8)** provides a synthesis of the main findings of Chapters 2 – 7 and considers the implications for fish farm management both in New Zealand and internationally. Recommendations are made about how enrichment effects can be most reliably quantified with particular regard to contrasting flow environments. A summary is provided regarding our ability to predict effects, and the discussion is extended to consider the relative merits of low and high flow sites in consideration of potential for wider ecosystem effects. The concept of recovery from such effects is then discussed in light of what was learnt, along with implications for farm fallowing and management. The Chapter concludes with some recommendations about where future efforts might be best placed in order to further advance our understanding of enrichment related effects.

CHAPTER 2

COMBINING BEST PROFESSIONAL JUDGEMENT AND QUANTILE REGRESSION SPLINES TO IMPROVE CHARACTERISATION OF MACROFAUNAL RESPONSES TO ENRICHMENT

Preface:

One of the first objectives of this research was to "evaluate existing approaches for assessment and prediction of ecosystem impacts". In order to do this, it was necessary to identify key distinguishing macrofauna species and the ecological significance/ function of those taxa. The aim was to then use that information to calculate a range a biotic indices, and alongside other more common or established indicators, to test their local relevance and applicability.

This Chapter therefore addresses the first stage of this objective and is a precursor to Chapter 3, which contrasts the resulting indices. It also introduces the Enrichment Stage gradient, which was developed specifically for this task and became an important underpinning framework for quantifying, defining and delineating enrichment throughout the thesis.

This work has been published in a refereed journal and has been adjusted to a standard format for the thesis, and as such there may be minor differences in the text, figures and tables compared with the published version. The citation for the original publication is:

Keeley N, MacLeod C, and Forrest B. 2012. Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment. Ecological Indicators 12, 154-166.

2.1 Abstract

Many benthic quality indices rely on categorising impacts by assigning species to ecological-groups (EGs) that reflect their tolerance to pollution. This is usually based on best professional judgement (BPJ) by experts with access to relevant ecological and taxonomic information. However, international applicability of such indices is restricted in areas where the species taxonomy, biology and response to pollution are poorly understood. In this study we describe an approach that enables objective allocation of EGs in situations where species information is limited. This approach utilised BPJ to categorise the environmental condition of benthic habitats around fish farms in New Zealand in relation to defined enrichment stages (*ES*s). Quantile regression was then used to model distributions of select taxa. The experts assigned *ES* scores from 1-7, for stations that ranged from relatively natural to excessively enriched (i.e. azoic), respectively, with judgements based on a suite of quantitative and qualitative indicators of enrichment, but without reference to detailed species information. The individual BPJ estimates were highly correlated, with minimal bias, indicating good agreement among the experts. Forty key indicator taxa were identified and quantile regression models based on *ES* (derived as a continuous explanatory variable) were fitted for 34. Abundances of the same taxa were also modelled in response to a more traditional enrichment indicator (organic content, %*OM*) for comparison with the BPJ technique. The regression approach characterised enrichment responses and objectively identified both the upper and lower tolerance limits of a range of taxa according to their *ES* and %*OM*. The models discriminated a number of key indicator taxa, including several that were responsive to low-level changes in *ES,* but not necessarily %*OM*. There was reasonable agreement (59%) between EGs derived using the regression approach and those defined using the AMBI database (one of the most commonly applied benthic quality indices). Moreover, the regression method allowed the classification of 10 additional taxa for which our ecological understanding was limited. A key outcome of this study was the acknowledgement that EG characterisations for species need to be regionally validated, no matter how well defined they might appear to be. The combined BPJ/ regression analysis approach described provides a valid means of both assigning and validating EG classifications, which will be particularly useful in situations where the taxa are poorly defined, and will enable existing biotic indices to be more broadly applied and interpreted.

2.2 Introduction

Physical and chemical changes to sediments beneath finfish farms can result in profound ecological effects (e.g. Brooks et al. 2002, Buschmann et al. 2006, Kalantzi & Karakassis 2006). Accordingly, in many countries environmental monitoring and assessment is undertaken to evaluate benthic conditions against environmental quality criteria. However, these quality criteria vary widely between locations and applications (Carroll et al. 2003, Kalantzi & Karakassis 2006), often relying on subjective expert opinion, also referred to as best professional judgement (BPJ, Weisberg 2008). Having a validated suite of standard metrics, cross-referenced with BPJ that can reliably define environmental quality would greatly improve our ability to compare both environmental effects, and management and regulatory responses across broad geographic regions.

Many ecological indices have been developed with a view to better informing BPJ; with several tested specifically for aquaculture-related benthic effects (Infaunal Trophic Index, Word 1978, e.g.: AZTI's Marine Biotic Index (AMBI), Borja et al. 2000, BENTIX, Simboura & Zenetos 2002, Multivariate-AMBI, Muxika et al. 2007). Of these, the AMBI was recently proposed as a primary indicator of biological health beneath finfish farms internationally (DSRSA, 2010). The AMBI (and related indices i.e. M-AMBI; BENTIX; MEDOCC, Pinedo & Jordana 2008) classifies benthic communities according to five ecological groups (EGs), based on their sensitivity to organic enrichment as defined by expert consensus (Borja 2004). Expert consensus, although critical, is a subjective step in the process, which can be time-consuming and requires an in-depth knowledge of responses of individual taxa to enrichment (or other forms of disturbance). Furthermore, incorrect assignment of species to Eco-groups (EGs) may result in misclassification of impacts (Simboura 2003, Borja 2004, Borja & Muxika 2005), and without site-specific validation, even closely related indices can imply a different quality status for the same site (Aguado-Gimenez et al. 2007). In a preliminary appraisal of the AMBI with data from aquaculture operations in New Zealand, we found only 29% of the 200 taxa identified were specifically listed in the AMBI database (AMBI v4.0, February 2010); the recommended minimum requirement is 80% for robust application (Borja & Muxika 2005). This highlights a major problem associated with the currently available suite of indices, which is how to deal with fauna that have a high degree of endemism and/ or which are poorly described. In many areas of the world the marine benthic fauna is still largely undescribed and as a result new locations will almost inevitably yield species whose response to enrichment is poorly understood.

In addition, macrofaunal responses to enrichment are generally complex, resulting from multiple biogeochemical and ecological interactions, and patterns are rarely adequately explained by a single continuous environmental variable (Borja et al. 2009b). Consequently, current statistical modelling approaches cannot readily incorporate the full suite of indicators used by experts to assess environmental quality in the BPJ process. Often relevant variables may be either deterministically qualitative or have responses where the outcomes cannot be interpreted independently of other variables. For example, although the mat-forming bacteria Beggiatoa spp. (Beggiatoa) can be a clear indicator of enrichment (Crawford et al. 2001, Macleod et al. 2004c, Hargrave et al. 2008), absence of Beggiatoa may reflect either a lack of enrichment, or conditions where enrichment is so severe as to limit this species (i.e. bottom-water is anoxic or Beggiatoa is disturbed by out-gassing). BPJ offsets these contradictions by taking into account all available information and interpreting indicators in the context of other measures of impact (e.g. Muxika et al. 2007, Weisberg 2008, Teixeira et al. 2010).

However, the challenge of quantifying the responses of individual taxa to the specified enrichment gradient remains. The basic premise behind this involves identifying the conditions (and the point along the gradient) at which the taxa is most prolific, i.e. its 'preferred' conditions. To do this, we adopted an approach successfully employed by Anderson (2008a), who modelled species distribution patterns in relation to sediment grain size using quantile regression splines, and derived preferences numerically. In this study we evaluated the effectiveness of BPJ combined with quantile regression (Cade & Noon 2003, Koenker 2005, Anderson 2008a) to define enrichment gradients using data from fish farms in New Zealand as a model. This dataset provided a full spectrum of enrichment (from natural to near-azoic) enabling identification of both the upper and lower tolerance limits of a range of taxa. By deriving BPJ as a continuous explanatory variable, the distribution of individual taxa was modelled across the enrichment gradient, enabling objective derivation of species into EGs. The aim of this study was therefore to test these methods as a means of evaluating enrichment sensitivities for poorly described taxa, and for assigning new, or validating existing EG classifications, thereby increasing the relevance and application of existing biotic indices to new regions.

2.3 Methods

2.3.1 Study sites, sampling and data selection

The data for this study were obtained as part of a regular compliance monitoring program for four Pacific salmon (*Oncorhynchus tshawytscha*) farms in the Marlborough Sounds, New Zealand (Figure 2-1). Sampling at each farm was undertaken annually in early summer (October-November) from 2001 - 2009. Although flow regimes varied slightly between farms, background environmental and operational conditions were comparable (Table 2-1). The analyses presented here were based on a subset of the full dataset, which deliberately encompassed a wide cross-section of annual feed inputs and associated levels of impact, and data that were consistent in sample size and distribution (i.e. no missing values for explanatory or derived biological variables; Table 2-1). The final dataset included all four farms (1 - 4 sampling occasions per farm), spanned nine years and resulted in 74 sampling stations consisting of 24 observations beneath cages, 38 along enrichment gradients adjacent to cages, and 12 at reference sites (Table 2-1).

Sediment samples were collected from directly beneath cages, and at stations along an enrichment gradient running away from the cages (25 – 250 m), as well as at references sites. Macrofauna were sampled using replicate (n = 3) perspex sediment corers (13 cm diameter, 0.0132 m2) deployed to a depth of 10 cm. Core contents were sieved to 0.5 mm, and the retained fauna identified and enumerated enabling calculation of a variety of community composition statistics (total abundance, number of taxa, Shannon-Weiner diversity (H') and Pielou's evenness).

The surface 3 cm of smaller composite sediment cores (7 cm diameter) was also collected for analysis of grain size and total organic matter (*OM*). Sediments were oven-dried to constant weight at 105ºC, and size class fractions from silt-clay through to gravel were analysed gravimetrically. *OM* was selected as a primary explanatory physico-chemical variable on the basis that large increases in *OM* can occur beneath salmon farms (e.g. Mazzola et al. 2000, Pohle et al. 2001) and that *OM* strongly influences benthic chemistry, metabolism and the associated macrofauna (e.g. Sampou & Oviatt 1991, Holmer & Kristensen 1992) . Percent *OM* was calculated as the % weight loss of dried samples after ashing at 550ºC for 2 h (modified after Luczak et al. 1997).

In addition observations were made of three qualitative indicators of enrichment from both sample cores and video footage of the seabed: (i) the presence of hydrogen sulphide odour - classified into one of five categories (none, mild, moderate, strong, very strong), (ii) coverage of *Beggiatoa* on the seabed - classified as none; patchy, up to 50%; extensive, > 50%, and (iii) presence and extent of out-gassing – classified as none, out-gassing on disturbance, freely out-gassing.

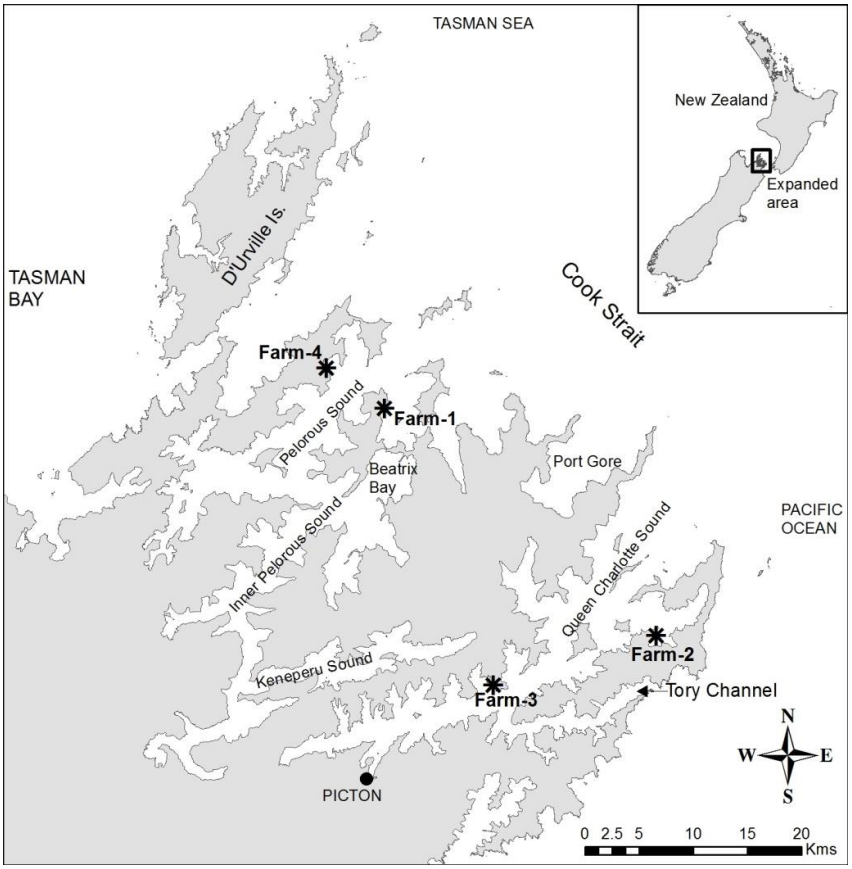

Figure 2-1: Map showing the position of the four salmon farms that comprised the study sites within the Marlborough Sounds, New Zealand.

Table 2-1: Summary of farm and environmental characteristics at the four study sites. OM = organic matter.

Site attributes	Values	Units	Farm-1	Farm-2	Farm-3	Farm-4
Year of surveys		20-	'01	'03,'05,'08,'09	'04,'06,'07	03,'05,'07,'09
Farm age at surveys		Years	7	14,16,19,20	19,21,22	14,16,18,20
Site depth		(range, m)	34-35	37-39	34-35	28-30
Current speed*	Mean(Max)	cm/s	3.1 (10.7)	6.0 (34.6)	3.7 (17.5)	8.4 (33.7)
Feed inputs		tonnes/yr	100-2264	1640-2239	2510-3289	2171-3918
Sampling stations		m from cages	0(×2), 25, 50, 75, 150, 250,Ref	0(×2), 50, 150, 250, Ref	0(×2), 50, 150, 250, Ctl	0(×2), 50×2, 100, Ref
Reference stations						
Sediment mud content	Mean(range)	%	84 (83-85)	55 (34-73)	80 (69-84)	78 (69-85)
%*OM*	Mean(range)	% w/w	4.9 (4.6-5.3)	5.0 (2.8-7)	5.2 (4.8-5.8)	4.9 (4.5-5.8)
All stations (incl. Reference)						
%OM	Range	% w/w	3.8-18	2.8-27	4.7-23	2.4-32
No. taxa	Range	No./core	1.6-32	2-30	1.5-26	1-36
Macrofauna abundance	Range	No./core	31-1012	3-2466	6.5-4230	1-4384
C. capitata abundance	Range	No./core	0-1010	0-1958	0-4209	0-2345

*Calculated for 20m water depth from 30-35 day deployments of Sontek ADCP positioned within c. 30 m of each farm.

2.3.2 Best professional judgement of enrichment status

Eight scientists with experience in the assessment of aquaculture impacts used BPJ to assign the 74 sampling events (station/farm/year) to one of seven enrichment stages (*ES*), based on narrative criteria (Table 2-2) adapted from previous studies (Pearson & Rosenberg 1978, Gray et al. 1979, Macleod & Forbes 2004). The seven *ES*'s described are specific for enrichment (rather than pollution or disturbance in general) and, importantly, take into account the full complement of indicator variables described above.

The experience of the BPJ group in assessment of aquaculture enrichment ranged from 2 - 35 years (mean = 13.5 years). Although seven of the experts were from the same institution (Cawthron Institute, New Zealand), significant bias was not expected, as high consistency in BPJ among international experts assessing benthic macrofaunal condition has been demonstrated by Texeira (2010). The eighth expert was from a comparable research institute (Tasmanian Aquaculture & Fisheries Institute) situated in Tasmania Australia. Furthermore, to minimise bias, agreement was sought among the experts on their understanding and interpretation of the

seven enrichment stages, in order to reduce the linguistic uncertainty inherent in the use of narrative criteria (Regan et al. 2002, Burgman 2005).

Each expert was provided with station-averaged physico-chemical data (depth, mean current speed, sediment grain size, %*OM*), the quantitative macrofaunal statistics and qualitative descriptors for each of the individual sampling events as described above), as well as the ranges (i.e. minimum, maximum) for each of the variables. The only species information provided was abundance of the globally ubiquitous, opportunistic polychaete, *Capitella capitata*, whose well-described response to organic enrichment was used to help define the enrichment gradient. Other taxonomic data was withheld to avoid confounding the results for later analyses, where individual species distributions were plotted against the enrichment gradient.

The BPJ assessment was blind with respect to site location and survey year, and the stations were listed randomly, so that experts did not bias results by anchoring on expected outcomes (e.g. the expectation of declining enrichment with distance from cage sites, Burgman 2005). The experts were asked to select the *ES* that 'best matched' the conditions for each station, but were also provided with a 'second best' option, for when conditions were deemed to fall between two *ES* stages. 'Second best options' were accounted for by adding or subtracting a nominal value of 0.3 (i.e. 30% of an *ES*) to the first choice scores, depending on whether the second choice was higher or lower than the first choice (respectively). Individuals' scores were collated and averaged; providing a continuous *ES* variable (between 1 and 7). The scores from each expert were plotted against the ranked averaged score and calculation made of the Spearman rank correlation coefficient (rho, ρ) of individual versus mean score (representing overall agreement), and the total deviation from the mean (td = $\sum(\bar{x}$ -x), representing overall bias).

Table 2-2: Narrative criteria describing seven enrichment stages, used by experts for best professional judgement (BPJ) assessments Modified from Macleod and Forbes (2004) and Pearson and Rosenberg (1978). Dominant ecological group refers to the AMBI Eco-group that was associated with the corresponding stage of enrichment for *ES* allocation.

ES	General description	Environmental characteristics	Dominant Ecological Group
1	Natural/pristine conditions	Environmental variables comparable to unpolluted/ un-enriched pristine reference site.	I
2	Minor enrichment: Low level enrichment. Can occur naturally or from other diffuse anthropogenic sources. 'Enhanced zone'	Richness usually greater than for reference conditions. Zone of 'enhancement' – minor increases in abundance possible. Mainly compositional change. Sediment chemistry unaffected or with only very minor effects.	(II)
3	Moderate enrichment: Clearly enriched and impacted. Significant community change has occurred.	Notable abundance increase, richness and diversity usually lower than reference. Opportunistic species (i.e. capitellids) begin to dominate.	III
4	Major enrichment 1:Transitional stage between moderate effects and peak macrofauna abundance. Major community change.	Diversity further reduced, abundances usually quite high, but clearly sub-peak. Opportunistic species begin to dominate, but other taxa may still persist. Major sediment chemistry changes.	IV
5	Major enrichment 2: Highly enriched. State of peak macrofauna abundance.	Very high numbers of one of two opportunistic species (i.e. capitellids, Nematoda). Richness very low. Major sediment chemistry changes. Bacteria mat (*Beggiatoa*) usually evident. H2S out-gassing on disturbance.	V
6	Major enrichment 3: Transitional stage between peak and azoic.	Transitional stage between peak and azoic. Richness & diversity very low. Abundances of opportunistic species severely reduced from peak, but not azoic. Total abundance low but can be comparable to reference. %*OM* can be very high (3-6 times Ref).	V*
7	Severe enrichment: Azoic/abiotic; sediments no longer capable of supporting macrofauna. Organics accumulating.	None, or only trace numbers of macrofauna remain. Some samples with no taxa. Spontaneous out-gassing; *Beggiatoa* usually present but can be suppressed. %*OM* can be very high (3-6 times Ref).	Azoic

*Note: EG V still dominant taxa, but conditions deteriorated beyond peak abundance.

2.3.3 Identifying key indicator taxa

A subset of taxa were selected (from a total of 139 taxa) for the application of quantile regression splines based on their dominance and power to discriminate effects. Reduction in the number of taxa focused efforts on those having the most influence on ecological index calculations, reduced the influence of uncommon species sampled by chance, and was necessary to make quantile regression analyses tractable. Dominance was evaluated based on total abundance and frequency of occurrence (i.e. sample count > 0) across the whole dataset. A multivariate canonical analysis of principal coordinates (CAP, Anderson & Robinson, 2003; Anderson & Willis, 2003) was used to model changes in community structure in relation to enrichment status as assessed by BPJ, to check for any taxa that were not otherwise notable in terms of abundance or frequency of occurrence. The CAP analysis was based on Bray-Curtis dissimilarities calculated from log-transformed abundances, using the PERMANOVA+ add-on for PRIMER v6 (Clarke 2006, Anderson 2008b). A check for over-parameterisation was conducted by choosing the number of PCO axes that minimised the leave-one-out residual sum of squares. A vector plot was overlaid onto the CAP to identify those taxa most strongly associated with the different stages of enrichment (Spearman correlation > 0.4).

2.3.4 Characterising the distribution of taxa along the enrichment gradient

Changes in distribution of each taxon among the 74 stations were characterised in response to the derived *ES* variable and compared to the more traditional measure of %*OM*. Using a method proposed by Anderson (2008a), abundances of each the indicator taxa were plotted against %*OM* or *ES* and quantile regression spline models constructed for the 95th percentile (Koenker et al. 1994, Koenker 2005), which represents the value below which 95% of the abundances are expected to fall (also called the $\tau = 0.95$ quantile). For five models (*Nucula gallinacean*, *Prionospio* sp., Cumacea, Melitidae and *Boccardia* sp. versus *ES*) the 90th percentile was used to reduce the influence of single outliers. In accordance with Anderson (2008a), all models were fitted using the function rq() combined with function bs() in R (R Development Core Team 2007). Polynomials of degree 2, 3, 4 and 5 were created for each taxon, with the best-fit model having the lowest value of the small-sample-correction version of Akaike's information criterion (AIC_c) (Cade et al. 2005, Anderson 2008a). If the next best AIC_c value was within 2 units of the chosen model but had a better visual fitted shape to the scatterplot of the data, then it

was chosen in preference (Burnham & Anderson 2002). The models were constructed on natural log-transformed abundances to minimise the tendency of the method to 'over-fit' the data (resulting in illogical curves), and converted back to raw abundances for plotting. Two exceptions to this were Ophiuroidea and *Cossura consimilis*, for which the raw data produced a more meaningful fit.

From each best-fit model, the value at which the predicted density achieved a maximum along the enrichment gradient identified the optimum *ES* or *%OM* for that taxon (X-optimum). Similarly, the peak abundance (Y-max) for each model was taken as the estimate of the maximum achievable density given optimum conditions. Y-max's were therefore estimated independently for both *ES* and *%OM,* and compared to test for consistency between the approaches and to provide confidence in the model outputs. Ninety-five percent bootstrap confidence intervals (e.g. Manly 2006) were obtained for both X-optimum and Y-max using bias-corrected percentiles from re-applications of the chosen model to each of 1,000 bootstrapped sample pairs.

2.3.5 Assigning Eco-groups

Eco-Groups (as per Grall & Glémarec 1997, Borja et al. 2000) were assigned for each taxon based on the abundance distributions and best-fit quantile regression splines in relation to ES. This was conducted on the basis that the dominant Ecological Groups (EG's) defined by Borja et al. (2000) correspond approximately to the first five Enrichment Stages (ES's), as they both reflect a progression from un-impacted conditions (ES 1 ≡ EG I) to highly impacted condi□ons where first order opportunists dominate (ES 5 ≡ EG V). Transi□onal stages were also similar, with the exception that EG II taxa are typically 'indifferent to enrichment' and may therefore also be present at higher levels of impact. Thus, for the purpose of this study we defined the correspondence between ES and EG as follows: ES 1-1.5 ≡ EG I, ES >1.5 – 2.5 ≈ EG II, ES >2.5 – 3.5 ≡ EG III, ES >3.5 – 4.5 ≡ EG IV, ES >4.5 ≡ EG V (Table 2-2). EG II therefore included taxa that were either most prevalent at ~ES 2, or proved indifferent to ES (i.e. occurred throughout ES 1-5 and showed no particular peak, making spline fitting difficult) and had low abundances. ES 6 and 7 are specific to organic enrichment; ES 6 represents a state beyond the 'peak of opportunists' defined by Gray (1979) and Pearson & Rosenberg (1978) but which is not yet 'azoic' (defined as ES 7).

2.4 Results

2.4.1 Best professional judgement

BPJ estimates from all eight experts were highly correlated with the mean (ρ = 0.917-0.975), indicating good agreement among individuals in assignment of each station to one of the seven stages of enrichment (Figure 2-2). The sum of the average deviations from the means (*td* values in Figure 2-2) was < 0.3 suggesting that overall the estimates were reasonably unbiased. Individual BPJ3 had the strongest bias of the experts, over-estimating *ES* by approximately one third of an enrichment stage (*td* = 0.29), whereas, BPJ2 tended to underestimate *ES* by 0.21 stages. Expert BPJ8 did not consider any sites to be clearly at *ES* = 1 (pristine) and hence scored all un-enriched sites as *ES* = 2, otherwise results were similar among individuals (inclusive of the Australian expert). Deviation from the mean was greatest in the mid-range of the *ES* gradient (3-5), peaking around *ES* 4.

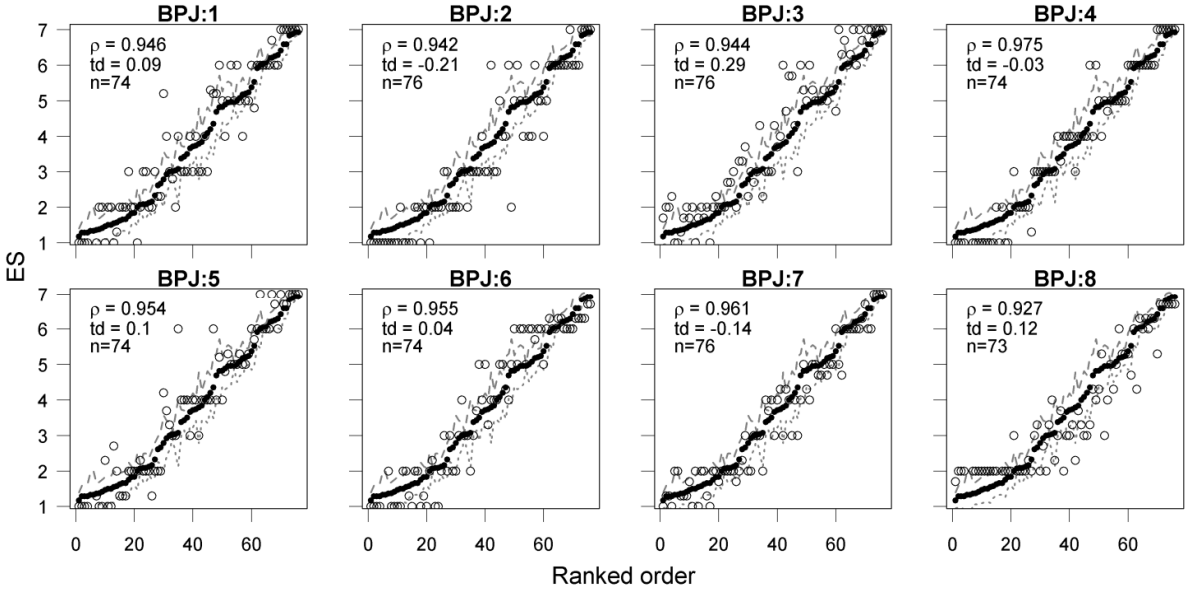

Figure 2-2: Results of best professional judgment assessment with individual responses (BPJ1-8, denoted by ○ compared to overall mean (denoted by ●). X-axis order by overall mean rank. Spearman rank correlation (ρ), total deviation from the mean (td) and sample number (n) indicated on top left of each plot. Grey dashed lines indicate 95th percentile for mean values.

2.4.2 Key discriminatory taxa

Forty one (41) potential indicator taxa were identified for further analysis, of which 35 were selected based on numerical dominance. Three taxa were selected on the basis that they occurred frequently but were not so abundant (*Cossura consimillus, Polynoidae, Flabelligeridae*), and two additional taxa (*Cadulus teliger* & Flabelligeridae) were identified from the CAP analysis (Figure 2-3). The taxa shortlist therefore included abundant opportunists, such as the polychaete *Capitella capitata*, less numerous, but commonly occurring taxa like Hesionidae, as well as any taxa that the CAP analysis suggested were associated with a particular *ES* (but were otherwise uncommon). Although not strictly macrofauna, Nematodes were included as they were frequently present, and often highly abundant in organically enriched sediments. Likewise, other taxa such as three amphipod families (Phoxocephalidae, Melitidae and Haustoriidae), a decapod (*Macrophthalmus hirtipes*) and the small arthropod, *Nebalia* sp., were considered potential indicators due to their prevalence, despite being relatively mobile surface dwellers.

CAP analysis (Figure 2-3) indicated *C. capitata* as the species most tolerant to enrichment, being associated with an *ES* of *5* to *6* (i.e. major enrichment) as determined by BPJ. Also tolerant, but more typical of *ES 4*, were Nematoda, followed by a mix of polychaetes, crustaceans and bivalves (Figure 2-3). Taxa associated with mildly enriched samples (c. *ES* 2) included the small bivalve *Theora lubrica*, and various polychaetes (Hesionidae, *Sphaerosyllis* sp. and *Prionospio* sp). Un-enriched sites (c. *ES* 1) were characterised by a range of polychaetes, ophiuroids, cumaceans and small molluscs (Figure 2-3).

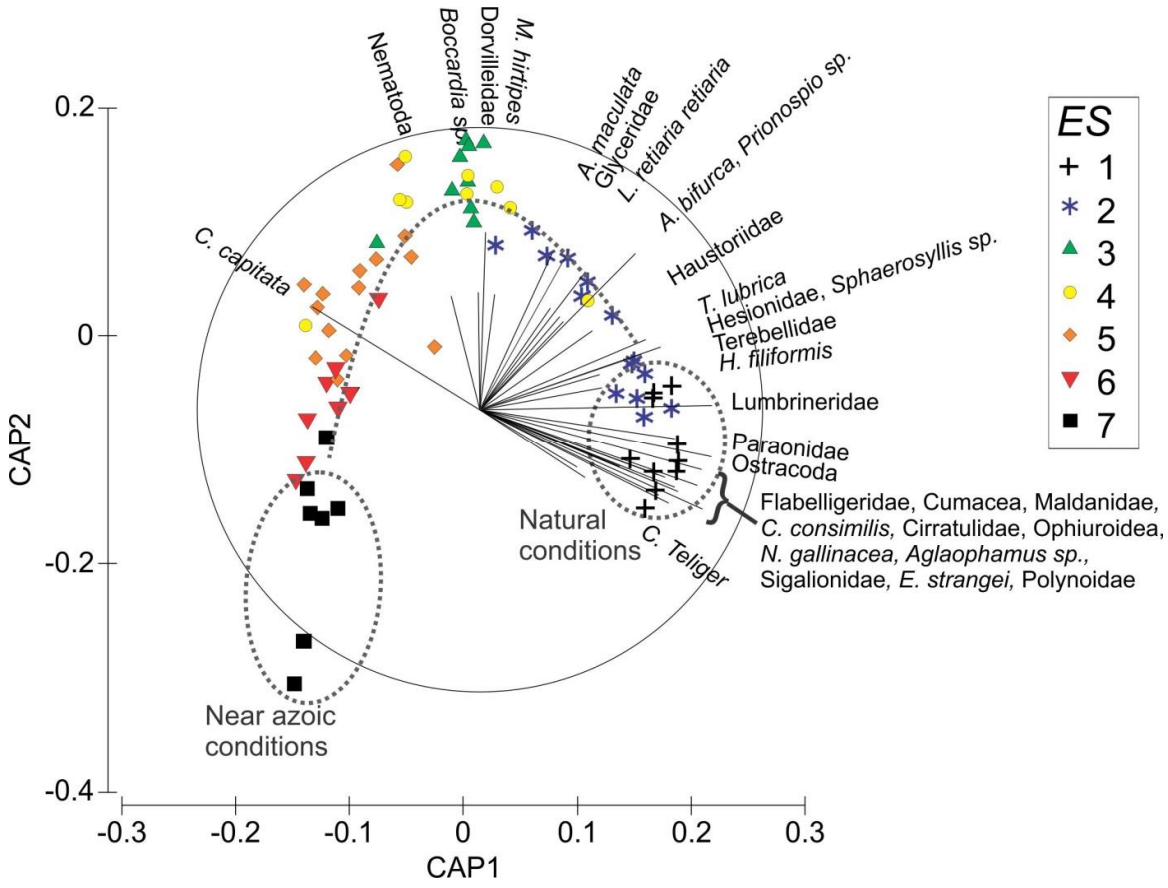

Figure 2-3: Canonical analysis of principle coordinates (CAP) of macrofauna data displaying maximum differences according to factor *ES*. For genus names see Table 3. The analysis was based on Bray-Curtis analysis of log-transformed mean abundances, using m=20 principal coordinates. Vector plot of correlated taxa (Spearman ρ >0.4) overlaid.

2.4.3 Quantile regression models of faunal responses

Best-fit 95th percentile regression splines revealed that the distribution of total macrofaunal abundance in relation to *ES* was unimodal and left skewed (Figure 2-4a). The modelled abundance peak occurred at *ES 5.1*, beyond which it declined sharply. The distribution of total macrofaunal abundance against %*OM* was also unimodal but in this case right skewed, with a modelled optimum peak abundance of 3,525 individuals per core at 9.1% *OM* (Figure 2-4b, Table 2-3). The number of taxa peaked between *ES 2* and *ES 3,* coincident with relatively low values of %*OM* (2.4-5%), and declined with increasing *ES* (Figure 2-4c) as %*OM* increased (Figure 2-4d).

Valid (biologically meaningful) regression models were able to be fitted for 34 of the 41 indicator taxa (Table 2-3, Appendix 2-A and 2-B). The method failed to fit meaningful splines for four taxa (Oligochaeta, *Polydora* sp., *Munna schauinslandii*, *Onuphis aucklandensis*) whose abundance and/or frequency of occurrence were too low, in which case enrichment sensitivity could not be evaluated and the taxa were omitted from the results. Similarly, meaningful models could not be fitted for *Heteromastus filiformis*, Terebellidae, Glyceridae and Tanaidacea. These taxa displayed no clear peak in relation to either *ES* or %*OM*, but such responses were biologically interpretable as indifference to enrichment, and the results have been included accordingly. For some distributions, the spline peaked at the X-minimum due to occasional high abundances occurring at or near the lowest X-values (e.g. %*OM* for *Sphaerosyllis* sp., Table 2-3). This latter pattern tended to have an adverse effect on the confidence intervals (bootstrapped estimates always equivalent to X-min) and the resulting X-optimum did not always fairly reflect the distribution of the data.

Estimated peak abundances (or Y-max) for each taxon spanned four orders of magnitude, from <10 (e.g. *Nebalia* sp. & *Aglaophamus* sp.) to 1000's (e.g. *Capitella capitata* & Nematodes). However, peak-abundance estimates derived independently in response to each of the explanatory variables (i.e. *ES* and %*OM*) were very comparable (raw abundance Spearman ρ=0.996, ln(abundance) Spearman ρ=0.976).

Models between taxon abundance and *ES* had a relatively wide distribution of data points across the explanatory axis (Appendix 2-A). Of the 34 taxa where the models were relevant, X-optimum and Y-max values identified four that could be considered indicative of highly enriched conditions (in order of reducing optimum-*ES*): *Nebalia* sp. (*ES* 4.8), *C. capitata* (*ES* 4.6), Nematoda (*ES* 4.1) and Dorvilleidae (*ES* 4.0) (Table 2-3, Figure 2-5a). However, the estimated

peak abundance for *Nebalia* sp. was 3 - 4 orders of magnitude lower than for the other three taxa. Taxa associated with moderate-high *ES* values were: Hesionidae and *Theora lubrica* (both *ES* 3.7), Glyceridae, Nemertea and *Boccardia* sp. (all *ES* 3.4) and *Armandia maculata* (*ES* 3.0). Several taxa were most abundant at an *ES* that corresponded to low to moderate levels of enrichment, including: *Sphaerosyllis* sp. and Phyllodocidae (both *ES* 2.9), two amphipod families (Phoxocephalidae, *ES* 2.9 and Melitidae, *ES* 2.8), *Arthritica bifurcata* (*ES* 2.8), Syllidae, *Prionospio* sp., *Leptomya retiaria retiaria* (all *ES* 2.5) and Paraonidae (*ES* 2.1).

Models between taxon abundance and %*OM* had a narrower distribution of data points across the explanatory axis than for *ES* (Figure 2-5b, Appendix 2-B). Of the 34 taxa with valid models, X-optimum and Y-max values identified those which were associated with a high organic content (Figure 2-5b, Table 2-3); many of which were also associated with higher ES values (Figure 2-6). These included: *Nebalia* sp. (14 % *OM*), *Capitella capitata* (12% *OM*), Nematoda (8.4%), *Boccardia* (8.4%), *Theora lubrica* (6.7%), Phoxocephalidae (5.9%), *Ennucula strangei* (5.4%), *Arthritica bifurcata* (5.4%) and Dorvilleidae (5.2%). The remaining taxa were generally associated with lower organic matter levels from 2.5% - 5% *OM* (Table 2-3, Figure 2-5b).

2.4.4 Comparison of ES and %OM, and definition of Eco-groups by regression

The level of rank correlation between *ES* and %*OM* was relatively low, although still significant (Spearman rank $\rho = 0.493$, $P<0.01$). The relationship was weakest at the early stages of enrichment, up to *ES* 3 (moderately-enriched) (Figure 2-6). Across these early stages, increasing *ES* values (indicated by the full suite of variables used by BPJ) were associated with faunal changes, without clear changes in %*OM* (%*OM* remained similar to background levels over this range of *ES*; Figure 2-6). Specifically, Hesionidae, Glyceridae, Nemertea, Phyllodocidae, Syllidae and *Sphaerosyllis* sp. were present when %*OM* was low, but where other environmental variables indicated moderate enrichment (e.g. changes in abundance and diversity). Thus, rank orders of Optimum-%*OM* and -*ES* over the mid-range of the scale were not well preserved. Beyond *ES* 3, %*OM* deviates markedly from background, opportunistic taxa begin to dominate, and others decline or disappear, as evident from both the CAP (see Figure 2-3) and regression model results. Hence, although models with *ES* and %*OM* defined a similar suite of faunal indicators at strong enrichment levels, *ES* was a more sensitive indicator than %*OM* alone.

Agreement between the EG classifications that were made based on estimated optimum-*ES,* and the 27 taxa that were also specified in the AMBI data base, was 59% (Table 2-3). Most of the taxa correspondence occurred at the genus or family levels (36% and 28%, respectively - many of which encompassed a range of species-specific EG's), and 28% were the same species. There were small disparities between the AMBI database and our classification in six taxa that differed by one EG (Glyceridae, Nematoda, Terebellidae and *Sphaerosyllis* sp., Polynoidae and Sigalionidae). More significant disparities existed between classifications for five taxa (i.e. *Armandia maculata*, Hesionidae, Cirratulidae, *Heteromastus filiformis* and *Cossura consimilis*), which differed by at least two EG's. In the present study *A. maculata* was found to occur under normal conditions, but was also tolerant of elevated sediment organic contents and *ES* 3, therefore this species was allocated an EG III, whereas in the AMBI data base it was classified as EG I. Cirratulidae were abundant under natural to moderately enriched conditions (*ES* 2 and low %*OM*) in the present study and were accordingly allocated an EG of II, compared with EG IV in the AMBI system. In the case of Nematoda, the AMBI data base suggests this group has an EG of III, whereas in this study Nematoda were absent below *ES* 3 and were believed to be more appropriately classified as EG IV based on an observed peak abundance at *ES* 4.1 and high *OM* (8.4%).

The regression spline method enabled us to identify enrichment stage preferences, and accordingly, EGs for 10 new taxa for which we had limited prior knowledge of sensitivity to enrichment (Table 2-3). These were *Arthritica bifurcata,* Cumacea, Dorvilleidae, Haustoriidae, *Leptomya retiaria retiaria,* Maldanidae, Melitidae, Ostracoda, Phoxocephalidae, and Paraonidae; most of which occurred in the early to mid-stages of enrichment (*ES* 1-3).

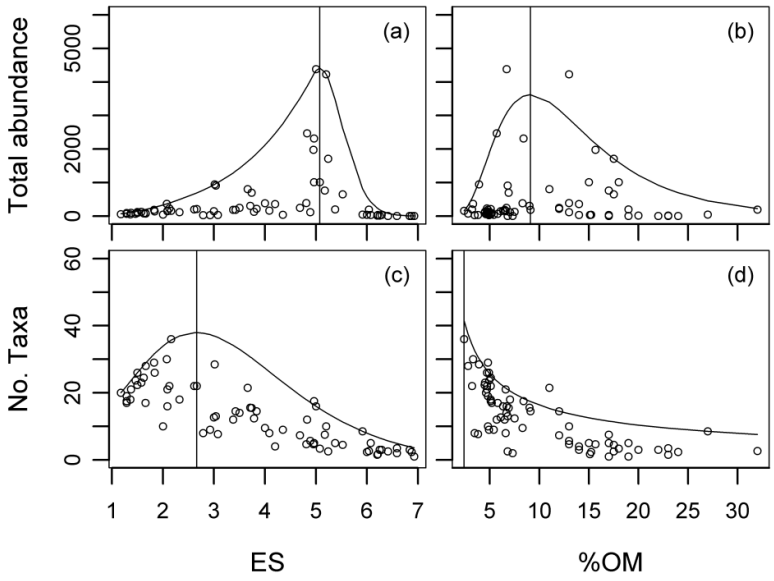

Figure 2-4: Relationships between total abundance with %*OM* and *ES* (a and b, respectively), and number of taxa with %*OM* and *ES* (c and d, respectively). Each point is an average of replicate cores from individual sampling stations. The regression spline for the 95th percentile, indicating the estimated optimum for the best-fit model, is shown by a vertical line.

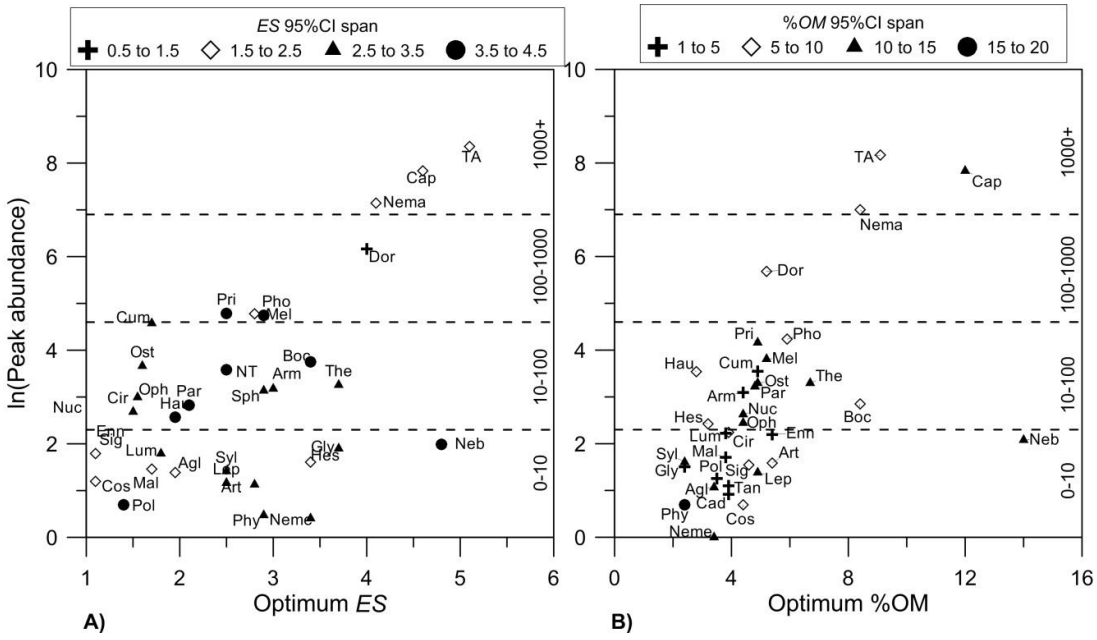

Figure 2-5: Plot of ln(estimated maximum likely abundance during optimum conditions) against estimated A) optimum *ES* and B) optimum %*OM* based on BPJ and regression spline analysis for 34 indicator taxa. Codes and names for taxa provided in Table 3.

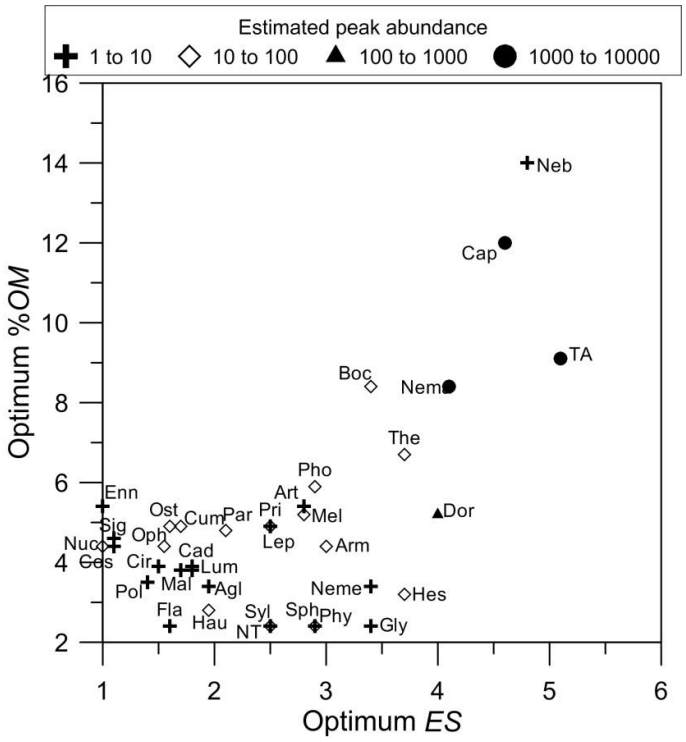

Figure 2-6: Plot of Optimum *ES* versus Optimum %*OM* based on BPJ and regression spline analysis for 34 indicator taxa. Spearman rank correlation (ρ) = 0.493 (P=0.002).

Table 2-3: The 37 indicator taxa along with taxonomic names and abbreviations used in text. Included are overall ranks in terms of abundance and frequency of occurrence, and a summary of results from quantile regression spline models indicating optimum-*ES* and -%*OM*, and peak abundance (Y-max) with 95 % CI (using indicated degree and d.f.). EG classifications assigned by regression using the *ES* variable are compared to the previously established AMBI EG's (AMBI v4 Feb 2010). Taxa groups: A, amphipod; B, bivalve; P, polychaete; O, ostracod; E, echinoderm; C, crustacean; N, nemertean. "Taxa level" defines the taxonomic level at which the comparison was made with the AMBI database (O, order, F, famiy, G, genus, s, species). Questionmarks (?) donote where the upper CI could not be reliably determined (see text).

			Rank:		**Optimum-%*OM***				**Opti-Y: max abund.**		**Optimum- *ES***				**Opti-Y: max abund.**		**Classifications**		
Taxa	Code	Taxa group	Abund.	Freq.	Deg	df	Peak	95CI	Ymax	95CI	Deg	df	Peak	95CI	Ymax	95CI	EG this study	EG AMBI database	Taxa Level
Total Abundance	TA	-	-	-	2	3	9.1	4.4-14	3525	2315-5203	2	4	5.1	3.6-5.8	4235	2850-1805	-		-
No Taxa	NT	-	-	-	2	3	2.4	2.4-2.4	40	37-91	4	3	2.5	1.0-4.6	36	26-48	-		-
Aglaophamus sp.	Agl	P	22	28	4	3	3.4	2.4-17	2.9	2.5-9	5	3	1.9	1.0-3.0	4.0	2.5-5	II	II	G
Armandia maculata	Arm	P	16	14	2	3	4.4	2.4-4.4	22	16-21	3	3	3.0	2.2-4.9	24	15-29	III	I	S
Arthritica bifurcata	Art	B	28	33	5	3	5.4	2.4-11	4.9	3.6-?	3	3	2.8	2.2-5.2	3.1	1.0-7.3	III	na	-
Boccardia sp.	Boc	P	42	8	2	3	8.4	5.2-10	17	17-48	5	3	3.4	1.6-5.4	42.5	12-?	III	I,II,IV	G
Cadulus teliger	Cad	Sc	60	63	4	3	3.9	3.0-6.1	2.5	-	4	3	1.8	1.0-3.8	2.4	-	II	II	G
Capitella capitata	Cap	P	2	1	2	3	12	12-22	2519	1205-4561	3	3	4.6	3.2-5.5	2527	1958-8989	V	V	S
Cirratulidae	Cir	P	14	19	3	3	3.9	2.4-10	9.4	5-	4	3	1.5	1.0-4.2	14.6	14-15	II	IV	F
Cossura consimilis	Cos	P	33	43	2	4	4.4	2.4-12	2	1.0-3.2	4	3	1.1	1.0-2.5	3.3	1.0-9.0	I	IV	S
Cumacea	Cum	-	10	9	5	3	4.9	3.3-5.7	35	34-?	3	3	1.7	1.0-3.6	97	11-175	II	na	-
Dorvilleidae	Dor	P	1	3	3	3	5.2	2.4-12	294	152-1371	5	3	4.0	3.8-5.2	474	140-2235	IV	na	-
Ennucula strangei	Enn	B	24	21	5	3	5.4	4.6-5.9	9	10-20	3	3	1.0	-	10	10-15	II	II	S
Flabelligeridae	Fla	P	36	50	4	3	2.4	-	2.1	-	5	3	1.6	1.0-3.2	-	-	II	I-II	G
Glyceridae	Gly	P	15	23	3	3	2.4	2.4-6.0	4.5	2.7-14	4	3	3.4	2.8-5.0	5.0	2.8-9.9	III	II	F
Haustoriidae	Hau	A	17	11	3	3	2.8	2.4-9.1	34	10.2-	2	3	1.9	1.0-4.6	13	3.0-43	II	na	-
Hesionidae	Hes	P	11	25	5	3	3.2	2.2-11	11	2.5-20	2	3	3.7	2.0-5.2	6.7	3.5-10	IV	II	F
Heteromastus filiformis	Het	P	18	13	-	-	-	-	-	-	-	-	Indif.	-	-	-	II*	IV	S
Leptomya retiaria retiaria	Lep	B	31	36	5	3	4.9	2.4-15	4.0	2.0-?	3	3	2.5	2.0-4.8	3.2	1.1-5.6	II	na	-

Table 2-3: *Continued…*

			Rank:		Optimum-%*OM*				Opti-Y: max abund.		Optimum- *ES*				Opti-Y: max abund.		Classifications		
Taxa	Code	Taxa group	Abund.	Freq.	Deg	df	Peak	95CI	Ymax	95CI	Deg	df	Peak	95CI	Ymax	95CI	EG This study	EG AMBI database	Taxa Level
Lumbrineridae	Lum	P	7	18	3	3	3.8	2.6-6.5	9.2	7-20	2	3	1.8	1.0-3.8	6.0	5.0-9.0	II	II	G
Maldanidae	Mal	P	25	29	4	3	3.8	2.4-5.7	5.5	3.5-8.8	5	3	1.7	1.0-2.8	4.3	3.6-6.4	I	na	-
Melitidae	Mel	A	4	7	3	3	5.2	2.4-15	45	40-161	3	4	2.8	1.6-3.8	119	22-529	III	na	-
Nebalia sp.	Neb	C	30	26	3	3	14	2.4-17	8	2.0-?	5	3	4.8	1.6-5.8	7.3	2.3-33	V	V	G
Nematoda	Nema	-	3	2	3	3	8.4	4.4-14	1093	509-2169	3	3	4.1	3.2-5.6	1262	480-9742	IV	III	P
Nemertea	Neme	N	26	30	2	3	3.4	2.4-15	1.0	3.0-10	2	3	3.4	2.0-4.7	1.5	1.0-4.0	III	III	P
Nucula gallinacea	Nuc	B	20	16	3	3	4.4	2.4-17	14	3.0-29	2	3	1.0	-	15	10-17	I	I	S
Ophiuroidea	Oph	E	19	17	2	4	4.4	2.4-14	11	-	5	3	1.5	1.2-4.0	20	7-41	II	II	C
Ostracoda	Ost	O	12	6	4	3	4.9	2.4-14	27	20-219	3	3	1.6	1.0-4.2	39	34-75	II	na	-
Paraonidae	Par	P	13	12	3	3	4.8	2-12	25	-	2	3	2.1	1.0-4.6	17	10-119	II	na	-
Phoxocephalidae	Pho	A	6	5	2	3	5.9	5.9-13	69	33-99	2	4	2.9	2.0-4.6	115	45-550	III	na	-
Phyllodocidae	Phy	P	27	32	2	3	2.4	2.4-18	2.0	1.0-10	2	3	2.9	1.6-4.6	1.6	0.8-8.5	III	II-IV	F
Polynoidae	Pol	P	32	41	4	3	3.5	2.4-3.8	3.5	3-6.5	5	3	1.4	1.0-3.0	2.0	1.4-6.6	I	II	F
Prionospio sp.	Pri	P	5	4	3	3	4.9	2.4-13	64	18-1015	4	3	2.5	1.0-4.6	120	30-200	II	II-IV	G
Sigalionidae	Sig	P	21	27	4	3	4.6	2.5-10	4.7	3.0-8.0	3	3	1.1	1.0-3.0	6.0	4.3-8.0	I	II	F
Sphaerosyllis sp.	Sph	P	9	15	2	3	2.4	2.4-2.4	21	21-72	4	3	2.9	1.6-5.0	23	7.0-54	III	I-II	G
Syllidae	Syl	P	38	35	3	3	2.4	2.4-17	5.0	-	4	3	2.5	1.6-5.0	4.1	0.5-7.0	II	II	F
Tanaidacea	Tan	C	29	34	2	3	3.9	2.4-7.5	3.0	-	-	-	Indif.	-	-	-	II*	II	O
Terebellidae	Ter	P	23	37	3	3	3.8	-	2.5	-	-	-	Indif.	-	-	-	II*	I	G
Theora lubrica	The	B	8	10	2	3	6.7	2.4-13	27	13-34	4	3	3.7	2.2-5.0	26	17-87	III	III	S

*Splines not able to be fitted to data, but visual interpretation of plots suggest indifference (= 'Indif.') to ES and OM and therefore EG = II.

2.5 Discussion

2.5.1 Best professional judgement

The strong correlations between individual best professional judgement (BPJ) assessments indicate that this is a reliable approach for evaluating enrichment status. Few analogous evaluations of BPJ have been undertaken to date, but this finding is consistent with a similar study undertaken by Weisberg (2008), in which experts ranked the environmental condition (from best to worst) for a wide range of marine sites. A more comprehensive assessment of *BPJ* consensus among regions also found good agreement among experts, regardless of country of origin (USA & Europe - Teixeira et al. 2010). Hence, in the present study average BPJ was considered a useful means of combining quantitative and qualitative information into a single continuous explanatory variable, enrichment stage (*ES)*.

However, difficulties in categorising stages were noted on a few occasions, i.e. when abundance and number of taxa were depleted and in combination with low (near background) %*OM* and moderate Shannon-Weiner diversity (*H')*. In these instances confusion can arise because of the potential for mixed response signals: low %*OM* frequently indicates low organic flux and un-enriched conditions (e.g. Cromey et al. 2002a, Callier et al. 2008), whereas very low abundances and number of taxa can be indicative of either early (pristine/natural) or late (near-azoic) stages of enrichment (Table 2-2). Teixeira et al. (2010) describe a similar situation with sites that had an unusually low number of taxa, and in that instance it was attributed to natural stresses associated with a higher energy environment. In our study some experts found that reviewing the broader suite of environmental parameters often clarified the situation, but that the *H'* index in particular was useful in distinguishing between conflicting conditions, especially when combined with specific signs of enrichment (e.g. H_2S odour or presence of *Beggiatoa*).

2.5.2 Biological patterns in relation to the explanatory variables

The observation that macrofaunal communities changed with increasing *ES* in the absence of a clear change in sediment *OM*, highlighted the greater sensitivity of *ES* as an explanatory variable. However, this is not entirely surprising given that *ES* integrates several quantitative and qualitative indicators. The absence of measureable changes in *OM* at early enrichment stages is consistent with a number of other studies that have also suggested *OM* is

a poor indicator of benthic condition, particularly at low levels (Hargrave et al. 1997, Mazzola et al. 2000, Macleod et al. 2004c). Such findings probably reflect the systems' capacity to assimilate and process *OM* at low influx levels. Nonetheless, degradation of organic matter in the early stages of enrichment can result in slight reduction of the sediments, which is better measured by alternative variables such as redox potential and sulphide concentration.

The relationships between total abundance and number of taxa with *ES*, and to a lesser extent %*OM* (see Fig 5), were consistent with ecological succession models for soft-sediments (Pearson & Rosenberg 1978, Gray et al. 1979, Glémarec & Hily 1981), and associated approaches to ecological classifications (Gray & Pearson 1982, Grall & Glémarec 1997, Borja et al. 2000, Rosenberg et al. 2004). There are also numerous case studies in the literature that support such models, in relation to organic inputs: (e.g. Nickell et al. 2003, Borja et al. 2009b); mussel farm enrichment, (e.g. Smith & Shackley 2004, Callier et al. 2007) and municipal discharges, (e.g. Taylor et al. 1998, Cardell et al. 1999).

Total abundance patterns and resulting optimum-*ES* assessments were strongly driven by densities of *Capitella capitata*. This species, as well as other taxa associated with high *ES* values (Nematoda, Dorvilleidae, *Nebalia* sp.), have all previously been identified as opportunists that are tolerant of salmon farm-related enrichment (Brooks et al. 2003, Edgar et al. 2005, Macleod et al. 2007, Tomassetti et al. 2009). The relatively small confidence intervals around abundance peaks for *C. capitata*, Nematoda and Dorvilleidae (see Table 2-3), suggest that these taxa are highly reliable indicators of the later stages of enrichment; although in the case of *C. capitata,* this result was anticipated due to its inclusion in the BPJ process. In contrast, *Nebalia* sp. was less abundant and had relatively wide confidence bands around its optimum *ES*. This suggests that while *Nebalia* sp. is tolerant of highly enriched conditions, it is not a true opportunist, as it does not necessarily proliferate under these conditions, and therefore is not as useful as an indicator species. The observed distribution pattern also explains why it was not discriminated by CAP analysis. Regression spline analysis appropriately identified taxa more commonly associated with mild to low enrichment, such as *Theora lubrica* and *Armandia* sp. (Edgar et al. 2005, Forrest & Creese 2006, Forrest et al. 2007) and indicators of un-enriched conditions such as *Ennucula strangei* and Maldanidae. This is consistent with the findings of Edgar et al. (2005) who described a strong negative association between *Ennucula* sp. and enriched sites, but is in contrast with Macleod et al. (2007) who suggested that Maldanidae were associated with moderate levels of organic enrichment. However, the

latter authors noted a proliferation of maldanids with organic enrichment only at a more exposed (higher flow) site, and the absence of maldanids under low enrichment conditions. They hypothesised that is was more strongly associated with resource limitation and the need for a reasonable supply of organic material, than with deteriorating environmental conditions *per se*. Finally, in the present study, Ophiuroids were identified as a good indicator of reasonably low (but sometimes mildly elevated) enrichment levels (*ES* 2); a finding that is consistent with other studies globally (Tasmania - Macleod et al. 2007, USA and Europe - Teixeira et al. 2010).

2.5.3 Quantile regression as a method to model faunal responses

Regression spline analyses effectively modelled the distribution of frequently-occurring taxa across the enrichment gradient, irrespective of abundance. A key advantage of this method is that it deals with the intrinsic asymmetry and non-linearity in the relationships between species distributions and environmental drivers, and also aligns directly with the ecological concept of limiting factors (e.g. excessive enrichment) acting as constraints on organism distribution (Thomson et al. 1996, Cade et al. 1999, Lancaster & Belyea 2006). These attributes were successfully exploited by Anderson, (2008a) to describe the responses of select benthic macrofauna to changes in sediment grain-size characteristics in a large estuarine data set. The present study further demonstrates the value of this approach, in this case for identifying and classifying macrofauna responses to an organic enrichment gradient.

Regression splines were particularly useful in distinguishing the response of taxa whose tolerance to enrichment was unknown or poorly understood by experts; in the present study, almost one third (10) of the taxa for which regression spline models were developed, and *ES* classifications determined, had no organic enrichment tolerance previously defined. Consequently, the approach appears well-suited to defining and validating key taxa for biotic indices; enabling inferences to be made regarding environmental tolerance and ecological succession stage, that could not have been readily achieved otherwise. The regression spline approach reliably defined enrichment associations of better known (and more dominant) taxa, thereby providing confidence in the classifications for these lesser known organisms. This enables classification of a subset of key taxa, that are important to the site(s) in question (and possibly lesser known), whilst still maintaining confidence in the index calculations as indicative of enrichment state. The method was less robust when applied to taxa with very

low abundance and frequency of occurrence, or whose distributions were poorly correlated with the explanatory variables, but, since uncommon species are not considered reliable indicators of environmental condition (Green & Young 1993), this should not be a major impediment to the application of this approach. Furthermore, it is especially valid when defining indicators of fish farm enrichment because in highly enriched situations, a small number of taxa can account for a high proportion of total individuals; in this study, 5% of the taxa accounted for just over 90% of the total abundance.

2.5.4 Comparison of quantile regression and Eco-group classifications

There was 59% agreement between the regression method and AMBI ecological group (EG) classifications and a further 22% only disagreed by one EG, which suggests that the BPJ/regression approach is a reliable means of determining EG's in situations where the fauna/ function is poorly known. However, it is important to note that several taxa deviated by two or more EGs between the BPJ/regression approach and the AMBI. For example, Cirratulidae EGs as determined by regression were classified two EG's lower than the stage indicated by the AMBI database (Grall & Glémarec 1997) and northern hemisphere experts (Teixera et al. 2010), who list Cirratulidae as equivalent to EG IV. In this instance, the disparity may be a function of endemic differences within the family grouping as disparities between locations at international scales are entirely plausible, especially were broad taxonomic groups are considered. For instance a single species within a group may be quite plastic, filling different ecological roles in different environments or modifying its role according to changing environmental conditions or resource availability (Jacobs & Podolsky 2010). Hence, grouping unclassified taxa to the next highest taxonomic level (e.g., Borja et al. 2008a) has the potential to be misleading; for example, in the AMBI database different species in the polychaete genus *Boccardia* span four of the five Eco-groups.

However, the inconsistency with overseas classification was also observed at a species level for three taxa. In the case of *Cossura consimilis,* this study identified it as being predominant under pristine conditions (classified as EG I) compared with a relatively high enrichment tollerace specified elsewhere (EG IV). The opposite was true for *Armandia maculata*, which occurred under moderately enriched conditions here, but is listed as EG I in the AMBI database. Finally, although the regression approach was not appropriate for taxa that appeared indifferent to enrichment (e.g. *Heteromastus filiformis*), such observations were

still meaningful with respect to the EG allocations, as EG II is partly defined by those taxa that are "indifferent to enrichment" (Borja et al. 2000). *H. filiformis* has been classified elsewhere as a second order opportunistic (EG IV; AMBI data base), which in New Zealand is consistent with the observation that it responds to disturbance generally rather than enrichment alone (Forrest & Creese 2006, Forrest et al, 2007). In our salmon farm case-study enrichment is considered the primary driver, which, based on the example of *H. filiformis*, may in part explain discrepancies with existing EG classifications.

2.6 Conclusions

The ability to apply and interpret ecological indices globally among locations requires a robust means of assigning Ecological Group (EG) classifications for poorly described taxa, as well as a means to validate previously defined taxa for different locations and environmental conditions. In this study, we combined best professional judgement (BPJ) and statistical modelling (quantile regression analysis) to objectively describe enrichment responses and test EG classifications on an organic enrichment dataset from New Zealand, which contained a number of poorly described taxa. The results confirmed 59% of the existing EG classifications, were similar on a further 22%, and provided a basis for assigning EGs for 10 new taxa. The work further highlights the importance of validating EG classifications when transferring and applying them internationally (Borja & Muxika 2005, Aguado-Gimenez et al. 2007). Accounting for and understanding the reasons for differences among locations will ultimately serve to improve the power and relevance of AMBI and other indices that use EG classifications. The evaluation approach (BPJ and statistical regression) proposed here can contribute to this goal in countries / regions where taxonomic and functional understanding of the fauna is incomplete.

2.7 Acknowledgements

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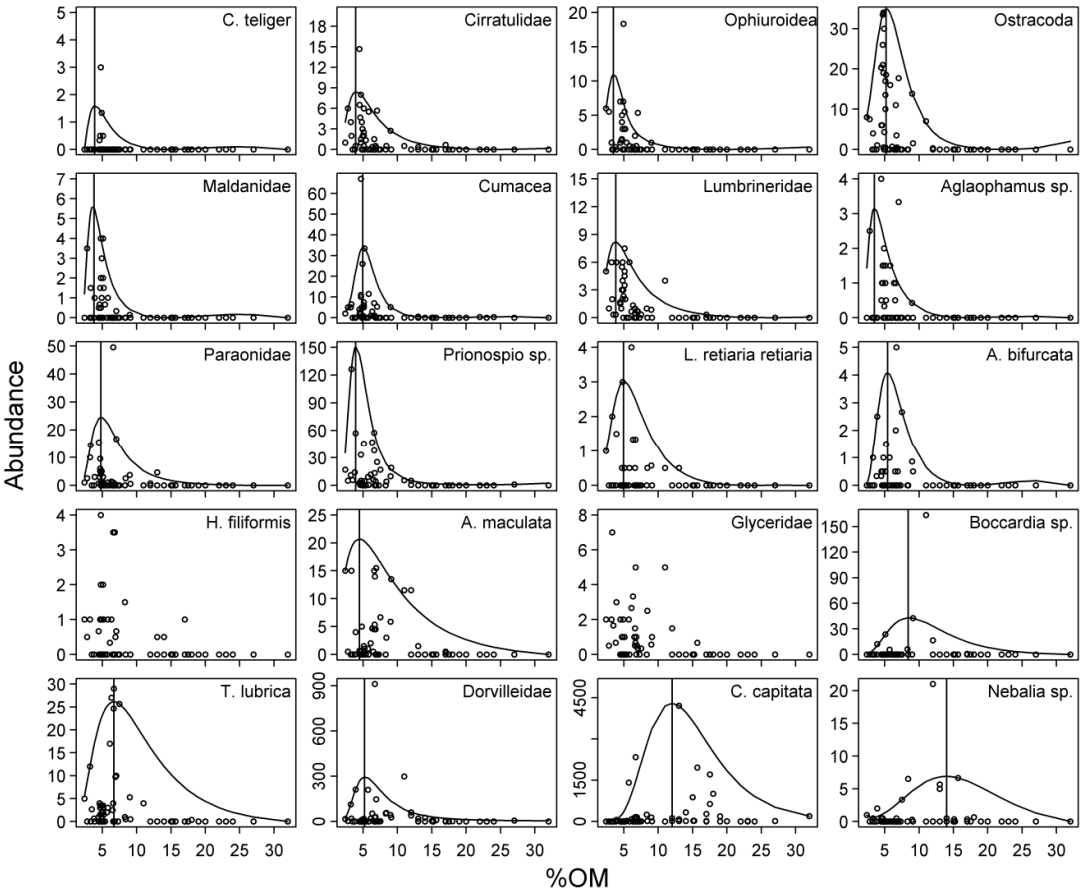
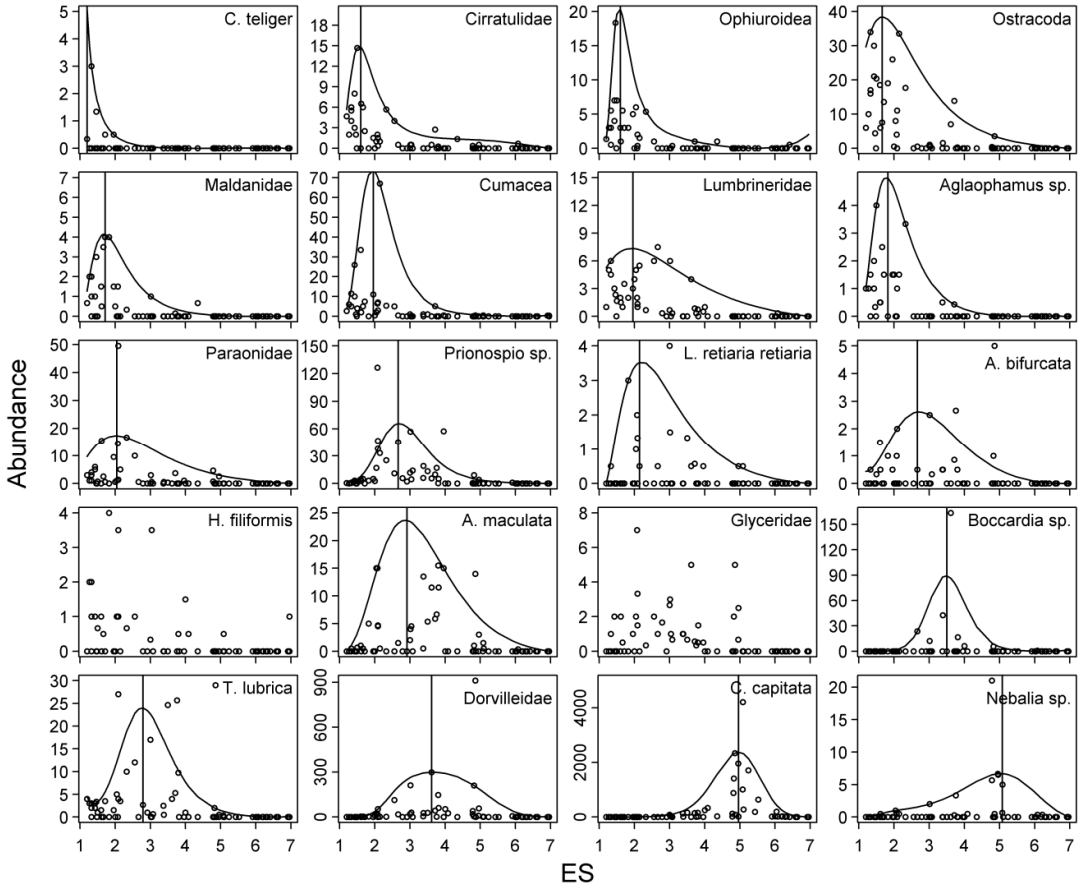
2.8 Appendix

Appendix 2-A: Relationship between 20 individual taxa (as indicated) and *ES* as assessed by BPJ. Each point is an average of replicate cores from individual sampling stations. The regression spline for the 95th percentile indicating the estimated optimum for the best-fit model for each taxon, is shown by a vertical line.



Appendix 2-B: Relationship between 20 individual taxa (as indicated) and %*OM* in sediments. Each point is an average of 2-3 cores from individual sampling stations. The regression spline for the 95th percentile indicating the estimated optimum for the best-fit model for each taxon, is shown by a vertical line.



CHAPTER 3

EXPLOITING SALMON FARM BENTHIC ENRICHMENT GRADIENTS TO EVALUATE THE REGIONAL PERFORMANCE OF BIOTIC INDICES AND ENVIRONMENTAL INDICATORS

Preface:

The motivation for Chapter 3 was to identify which variables, or suite of variables, best characterise enrichments effects across the full spectrum of enrichment, with emphasis on versatility and their ability to provide relevant impact classifications in different flow environments. The need for versatility with respect to different flow environments was considered important, as preliminary observations suggested that the way in which the benthic ecology responded to enrichment was different to that commonly described for the more traditional and well-studied "low flow". This Chapter therefore contributes to the gap in understanding that exists around how high flow sites by empirically describing the relationships between common infaunal and physico-chemical variables and contrasting how they respond to enrichment. This provided a quantitative means of determining overall Enrichment Stage and identified the most reliable variables are identifying for use in later chapters; for example, when predicting effects from depositional models in Chapter 5 and describing recovery patterns in Chapters 6 and 7.

This work has been published in a refereed journal and has been adjusted to a standard format for the thesis, and as such there may be minor differences in the text, figures and tables compared with the published version. The citation for the original publication is:

Keeley N, Forrest B, Crawford C, and Macleod C. 2012. Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators. Ecological Indicators 23: 453-466.

3.1 Abstract

This study evaluates five benthic indicators (total abundance, number of taxa, redox potential, total free sulfides, total organic matter) and ten biotic indices (Margalef's d, Peilou's J', Shannon H', AMBI, M-AMBI, MEDOCC, BENTIX, BOPA, ITI, BQI), to identify those that best define organic enrichment gradients under different flow regimes. Performance was measured against Enrichment Stage (ES), a continuous variable characterising the full range of sediment conditions (natural to azoic). None of the 15 metrics were able to consistently discriminate over the full enrichment gradient for both flow environments. The most versatile indices were BQI > M-AMBI > AMBI > Log(N) > BENTIX. Of these, M-AMBI best catered for different flow environments, while the BQI was the most effective under highly enriched conditions. Under strong enrichment, i.e. when macrofauna abundance is in decline, changes in redox, sulfides, number of taxa and abundance were reasonably clear. However, the more complex biotic indices were relatively insensitive at this level, highlighting a limited applicability beyond the 'peak of opportunists' (PO). Conversely, in high flow regimes, some of the biological indicators were relatively sensitive to low-to-moderate levels of enrichment that were not well discerned by the physico-chemical variables. A useful subset of variables for assessing enrichment status is recommended, comprising two of the best performing biotic indices that are based on alternative/ independent classification schemes (i.e. EG's and $ES50_{0.05}$), total abundance, to aid in discerning PO, and a geochemical variable (redox or S^{2-}). Inconsistencies between metrics were found to be more significant than the variability surrounding the predictive capacity of individual indicators, and as a result there is a risk of ES misclassification where only a single index is used. Whilst there is a recognised need to use combinations of indicators, this study also stresses the importance of focusing on a few regionally validated measures and down-weighting the importance placed on any that are not. Additionally, although using a combination of different indicators may produce a 'safe' average result, it may be inefficient, and the averaging effect has the potential to mask extreme conditions. Hence, there remains a need for expert judgement to select and appropriately weight indicator variables, to identify any erroneous results, and to reliably assess ecological quality status.

3.2 Introduction

Many biological indicators have been proposed for characterising anthropogenic impacts on soft-sediment benthos; most of which have as an ecological foundation the Pearson & Rosenberg paradigm that depicts community response to gradients of organic pollution or disturbance (Pearson & Rosenberg 1978, Glémarec & Hily 1981). These range from relatively simple, long-standing diversity or dominance measures such as species richness, Pielou's evenness and Shannon-Weiner diversity; to more complex multi-metric indices such as the benthic quality index (Rosenberg et al. 2004), and the multivariate-AZITES Marine Biotic Index (M-AMBI, Muxika et al. 2007). The proliferation of different indices has arisen because of a natural inclination to develop site and situation specific measures, using endemic taxa, locally favoured variables and local knowledge. As it would be highly beneficial to have more widely applicable indicators, it is important that we evaluate the suitability of those that already exist, before developing new ones (Peet 1975, Diaz et al. 2004, Borja & Dauer 2008).

A variety of indicators have been tested for their utility in assessing the benthic effects of shellfish and finfish aquaculture (Salas et al. 2006, Bouchet & Sayriau 2008, Callier et al. 2008, Borja et al. 2009b, Edgar et al. 2010), with much effort being put into testing ecological quality statuses (EcoQS) inferred by select indices (e.g. the European Water Directive Framework, Borja et al. 2003, Rosenberg et al. 2004, Borja et al. 2008b). Several of the main indices have been summarised and compared to facilitate their use (Salas et al. 2006, Pinto et al. 2009, Ranasinghe et al. 2009); however, there are also acknowledged limitations regarding their transferability across different spatial or temporal scales, water depths, and habitat or substratum types (Reiss & Kroncke 2005, Aguado-Gimenez et al. 2007, Borja & Dauer 2008). Therefore, the need to identify measures that are universally applicable and able to integrate across different ecosystem types (Borja et al. 2009a) still exists, and needs to be expanded to include comparisons of the performance and comparability of different indices.

Marine aquaculture installations provide useful model systems in which the effectiveness of benthic ecological indicators can be evaluated under very defined conditions. Seacage finfish farming is typically conducted in environments where other anthropogenic stressors (e.g. pollution) are minimal, and there are typically multiple farms within a region; thus providing the conditions for a robust comparison of different metrics at a regional-scale.

Moreover, the benthic enrichment gradient that arises in the vicinity of finfish cages is generally strongly-defined across small spatial scales (tens of metres), often with a full spectrum of conditions from anoxic/azoic to natural. Along the enrichment gradient, the "peak of opportunists" (PO) is a defined point (Pearson & Rosenberg 1978) that has explicit indicators and index values. However, the ecological characteristics underpinning the progression from PO to azoic conditions associated with extreme enrichment are less clear, can create computational difficulties for indices, and as a result, are often simply represented by a categorical shift (e.g., Majeed 1987, Simboura & Zenetos 2002, Dauvin & Ruellet 2007). Nonetheless, the transition from PO to azoic (i.e. where densities of opportunists start to decline) is a threshold commonly used in assessing and managing effects of seacage fish farms worldwide (Wilson et al. 2009). Increasingly, the ecological effects of finfish farms are also managed by imposition of maximum limits on the spatial extent of measurable effects, which requires the ability to detect the point at which benthic conditions differ from background. Hence, to be widely applicable and useful in this context, it is important for indicators to be able to quantitatively characterise conditions at both ends of the enrichment spectrum. Moreover, the indicator response to a given level of enrichment should ideally be consistent across a range of environmental conditions.

In addition to biological measures (Pinto et al. 2009, Edgar et al. 2010), the many indicators that have been used to define seabed enrichment include geochemical measures (Hargrave et al. 2008, Hargrave 2010) and even visual condition of sediments (Crawford et al. 2001, Macleod et al. 2004a). However, only a limited number of studies have attempted to integrate such measures (Macleod et al. 2004a, Hargrave et al. 2008, Keeley et al. 2012b) and it is evident that benthic assemblages are seldom readily correlated with a single explanatory environmental variable for enrichment (Borja et al. 2009b, Keeley et al. 2012b). Furthermore, there is a need to quantitatively synthesise conflicting interpretations, such as when different measures give different impressions of benthic quality status (Aguado-Gimenez et al. 2007), or show site-specific variation in their responses to enrichment (Pohle et al. 2001, Macleod et al. 2007). One way of addressing these issues is to incorporate inferences from multiple indicators (both qualitative and quantitative) using best professional judgement (Weisberg 2008, Teixeira et al. 2010). This approach was employed recently for salmon farms in New Zealand by Keeley et al. (2012b) to derive a bounded, continuous explanatory variable

"enrichment stage" (ES, on a gradient from ES1: natural to ES7: anoxic/azoic) that reliably predicted the enrichment responses of soft-sediment infauna.

In this study, we evaluate the performance of different indicators and indices against the ES variable, to identify those that best define the full spectrum of enrichment that occurs beneath salmon farms in our study region. Indicator performance is also compared across two contrasting water current flow regimes (low and high flow as defined below), as previous research has shown that the environmental impacts (both biological and chemical) will vary according to the prevailing hydrodynamic conditions (Maurer et al. 1993, Macleod et al. 2007). The relationships between infaunal response and the associated physico-chemical reaction to enrichment were evaluated, with respect to these regionally relevant criteria, for a suite of environmental indicators and commonly reported biotic indices. This provides a comprehensive assessment of the versatility of the respective indices and an evaluation of their ability to provide an ecologically relevant classification of both enrichment level and ambient environmental conditions.

3.3 Methods

3.3.1 Study sites and datasets

The study uses subsets of data from a 12 year (1998 – 2010) dataset of annual monitoring at five salmon farms (27 – 40 m depth) aged between 1 and 22 years (Table 3-1), located within the Marlborough Sounds, New Zealand (Figure 3-1). Three of these farms (A – C) had mean current velocities of < 9 $cm \cdot s^{-1}$ at 20 m water depth (approximately mid-water) and are hereafter referred to as 'low flow' sites, whereas the other two (D and E) had velocities of > 15 $cm \cdot s^{-1}$, and are referred to as 'high flow' sites. All of the sites are situated over soft sediments, but the low flow sites tended to be sandy-mud (55 – 80 % mud), whereas the sediments at the high flow sites comprised muddy-sand (28 – 32 % mud; Table 3-1).

As not all measured response variables were recorded at all times, subsets of the data were extracted for analysis. The first dataset spanned seven years (2003 to 2009) and included data from 16 different surveys (year-farm combinations) representing a broad cross-section of feed/ production levels (1640-4120 $t \cdot yr^{-1}$) and therefore, presumably, of benthic effects (Table

3-1). Farm-specific information was collected for each sampling site and included: recent feed usage ('Feed', total tonnes for 6 months prior to sampling), farm age at sampling ('Age', years), average current speed ('Current', $cm \cdot s^{-1}$; measured at ~20 m water depth from 30 – 35 day current meter deployments); and sampling station properties: depth ('Depth', m), distance from farm ('Distance', m), sediment grain size distribution (utilising % mud content), percent organic matter (% ash free dry weight, Luczak et al. 1997), and infauna composition and abundance. Also included were qualitative measures of sediment odour ('Odour'), bacterial mat coverage (Beggiatoa) and sediment out-gassing, these were evaluated in the field using pre-determined categories described in Keeley et al. (2012b). A second dataset contained data from the same five farms, but only covered the period 2009 –2010, as in these years total free sulfides (S^{2-}, µM) and redox potential (Redox, Eh_{NHE}) were also measured. For both subsets of data, analyses were conducted on station-averaged values, generally derived from triplicate samples.

The sampling sites at each farm were originally positioned to evaluate the spatial extent of effects (relative to the cages) and typically included two beneath-cage sites, two or three sites at increasing distances away (up to 250 m) and reference sites at least 1 km from cages (Table 3-1). The sites were located in the field using GIS and an on-board mapping system. The seabed was sampled with a Van-Veen grab and the macrofauna and sediment physico-chemical properties were subsampled following the methods of Keeley et al. (2012b). Macrofaunal (organisms retained on a 500 µm mesh) abundances were based on counts from core samples (13 cm deep × 10 cm diameter, 0.0132 m^2).

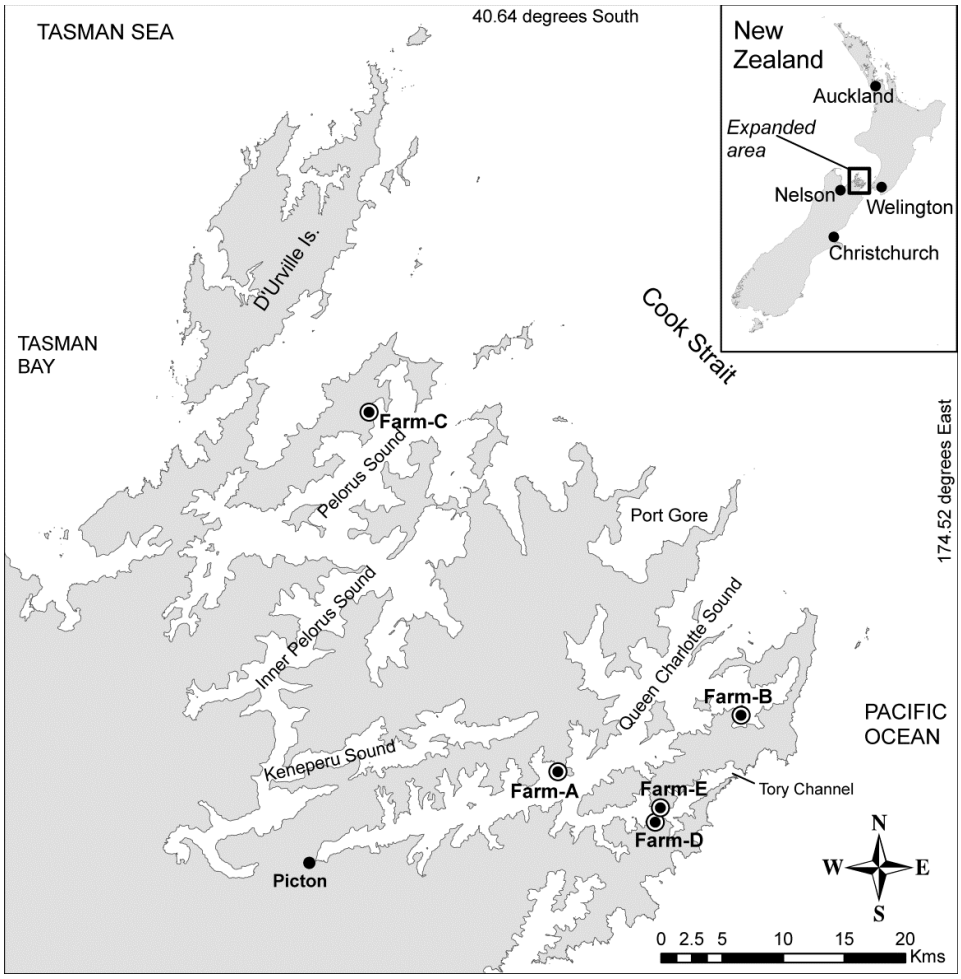


Figure 3-1: Location of study sites, Marlborough Sounds, New Zealand.

Table 3-1: Summary of farm and environmental characteristics at the five study sites. OM=organic matter.

Site Attributes	Values	Units	Farm-A	Farm-B	Farm-C	Farm-D	Farm-E
Year of survey		20-	'04,'06, '07	'03,'05, '08,'09	03,'05, '07,'09	'03,'05, '08	'08,'09
Farm age at survey		Years	19,21,22	14,16, 19,20	14,16, 18,20	11,13,16	1,2
Site depth	Range	m	34-35	37-39	28-30m	27-31	30-40
Current speed*	Mean(max)	$cm \cdot s^{-1}$	3.7(17.5)	6(34.6)	8.4(33.7)	15(55.9)	19.6(109)
Feed inputs	Range	$mt \cdot yr^{-1}$	2510-3289	1640-2239	2171-3918	2104-4120	2631-3526
Sampling stations	Distance from cages	m	0(×2),50, 150,250, Ref	0(×2),50, 150,250, Ref	0(×2), 50(×2), 100, Ref	0(×2),50, 100, Ref	0(×2), 50(×2), 100(×2), 150(×2), 200(×2), 250(×2), Ref
Reference stations							
Sediment mud content	Mean(range)	%	80 (69-84)	55 (34-73)	78 (69-85)	28 (21-38)	32 (29-37)
%*Organic matter*	Mean(range)	% w/w	5.2 (4.8-5.8)	5.0 (2.8-7)	4.9 (4.5-5.8)	3.1 (2.5-3.7)	3.3 (2.5-4.2)
No. taxa	Mean(range)	No./core	22 (18-28)	18 (17-19)	20 (16-23)	35 (27-48)	39 (31-42)
Macrofauna abundance	Mean(range)	No./core	107 (76-147)	72 (52-92)	78 (37-128)	218 (152-285)	231 (102-278)
Near-farm stations							
%*Organic matter*	Range	% w/w	4.7-23	3.3-27	2.4-32	1.6-5.8	2.3-9.1
No. taxa	Range	No./core	1.5-26	2-30	1-36	8.5-36	8-54
Macrofauna abundance	Range	No./core	6.5-4230	3-2466	1-4384	210-6,900	81-20,000
C. capitata abundance	Range	No./core	0-4209	0-1958	0-2345	1-2870	0-15,100

3.3.2 Best professional judgement (BPJ) of enrichment stage

For both datasets, which comprised 117 sampling events (year-farm-sampling site combinations; 70 low flow, 47 high flow), six scientists experienced (mean 13.6 years) in the assessment of aquaculture impacts used BPJ to assign each sampling station (based on station-averaged data) to one of seven enrichment stages (ES) according to methods in Keeley et al. (2012b). The seven ES's are specific for organic enrichment and integrate a suite of indicator variables. The first five ES's correspond to the Eco-Group's (EG's) defined by (Grall & Glémarec 1997) and (Borja et al. 2000), in that they reflect a progression from unimpacted conditions (ES 1/ EG I) to highly impacted conditions where first order opportunists dominate (ES 5/ EG V). ES 6 and 7 are specific to organic enrichment beyond that catered for by EG classifications; ES 6

represents a state beyond the 'peak of opportunists' (Pearson & Rosenberg 1978, Gray et al. 1979) but which is not yet 'azoic', (the latter is defined as ES 7). The station-averaged dataset provided to each expert consisted of physico-chemical data, species composition data and associated macrofaunal statistics (total abundance, N; number of taxa, S), and qualitative descriptors of enrichment as described by Keeley et al. (2012b). The experts' scores were averaged to produce a bounded continuous variable across ES 1 – 7.

3.3.3 Selection and calculation of biotic indices

Of the many indicators available, ten biotic indices and four other variables (Table 3-2) were selected based on the following criteria (in reducing order of importance): i) suitability for organic enrichment (as opposed to pollution or disturbance more generally) in marine systems (i.e. with constant salinity), ii) generality of use and demonstrated applicability to effects from aquaculture, and iii) calculation simplicity and track record of use. Margalef Index, Pielou's *J'* and Shannon *H'* were calculated using the DIVERSE function in PRIMER v6; the remaining indices were calculating according to the established methods outlined in the relevant references (Table 3-2). Reference conditions used to calculate M-AMBI were determined separately for high flow and low flow sites using a historical data-based method (Borja et al. 2012) due to the availability of a robust, regionally and temporally relevant dataset. This involved compiling information from appropriate reference sites (i.e. similar depths, substrates and sampled during the previous 12 years) situated within the Marlborough Sounds (n = 99 and 50, low and high flow sites respectively), and ranking them according to their ecological quality status inferred from values of: i) S, ii) *H'* and iii) AMBI (Borja 2004), and then according to the sum of those ranks. Reference conditions for M-AMBI were then determined by taking the average of the top five (i.e. least impacted) samples for each variable, when ordered according to the sum of the ranks. Using this approach, the following reference conditions were derived; low flow: S = 26.2, H' = 2.59, AMBI = 1.09; high flow: S = 45.2, H' = 3.04, AMBI = 1.83.

The AMBI, M-AMBI, MEDOCC (MEDiterranean OCCidental index) and BENTIX (Benthic Index) all utilise species classification according to the five EG's (Table 3-2). Although there is an EG database that classifies a broad range of taxa (http://ambi.azti.es), many of the taxa in

this study were yet to be classified. In these instances EG's were assigned based on a combination of local observations (following the methods of Keeley et al., 2012), and relationship to "nearest neighbour" classifications. The Infaunal Trophic Index (ITI) was calculated by assigning taxa to functional feeding groups, based on published information and expert advice from local taxonomists. The Benthic Quality Index (BQI) allocates a sensitivity/tolerance score for taxa: $ES50_{0.05}$, which is the expected number of species (ES) among 50 individuals according to Hurlbert's (1971) formula (Table 3-2). The subscript denotes that the 5th percentile is selected to indicate the species tolerance value (see Rosenberg et al., 2004). $ES50_{0.05}$ values were calculated for 743 taxa from the region, comprising 4,426 infaunal samples collected from a variety of impacted and reference sites over the 12 year period. The values that were determined for the 50 most frequently occurring taxa are provided in Appendix 3-A.

Table 3-2 (overleaf): Equations used to derive environmental indicators and biotic indices with summary of established (published) classification schemes and associated category boundary values for each of the environmental indicators/indices that have been utilised in Figure 5. na = not applicable.

Source references: 1: Hargrave et al. (2008); 2: Brooks et al. (2003); 3: Margalef Margalef (1958); 4: Pielou (1966); 5: Schaanning and Hansen (2005); 6: Hansen et al. (2001); 7: Borja et al. (2000); 8: (Borja, 2004); 9: Muxika et al. (2007); 10: Simboura and Argyrou (2010); 11: Pinedo & Jordana (2008); 12: Word (1978); 13: Rosenberg et al. (2004); 14: Simboura and Zenetos (2002); 15: Dauvin and Ruellet (2007); 16: Cromey et al. (2002); 17: Chamberlain and Stucchi (2007); 18:Hurlbert (1971); and 19 Leonardsson et al. 2009.

Variable	Classification type	Formulae & established clasification schemes & boundary values	Source
Redox			
Enrichment zones:		Oxic-A / Oxic-B / Hypoxic-A / Hypoxic-B / Bad > 100 / -50 / -100 / -150 / <	1
Sulphides			
Enrichment zones:		Oxic-A / Oxic-B / Hypoxic-A / Hypoxic-B / Bad < 750 / 1500 / 3000 / 6000 / >	1
Total abunance (N)		= average number of individuals per 13 cm diameter core	na
No Taxa (S)		= average number of taxa per 13 cm diameter core	
Biodiversity indicator:		High / Moderate / Reduced / Very low	1(2)
(% reduction)		<25% / 45% / 59% / 85% / 98%	
Margalef's richness (*d*)		= (S-1) / log N	
		Limitless scale based on S and N - index usually between 0 (low) and 10 (high).	3
Pielou's evenness (*J'*)		= *H'* / log S	
Nominal linear scale:		High / Bad 1 / 0.8 / 0.6 / 0.4 / 0.2 / 0	4
Shannon Diversity (*H'*)		= $-\sum_i p_i \log(p_i)$ where p is the proportion of the total count arising from the *i*th species	
Biodiversity indicator:		High / Moderate / Reduced A / Reduced B / Very low > 4 / 3 / 2 / 2 / 0	1(5,6)
Equivalent EcoQS:		High / Good / Moderate / Poor / Bad > 5 / 4 / 3 / 1.5 / 0	14
AMBI		= [(0 × %GI + 1.5 × %GII + 3 × %GIII + 4.5 × % GIV + 6 × %GV)]/100 where GI, GII, GIII, GIV and GV are ecological groups (see Section 2.3).	
Pollution classification:		Unpolluted / Slightly / Meanly / Heavily / Extremely 0 / 1.2 / 3.3 / 5 / 6 / 7	7
EcoQS:		High / Good / Moderate / Poor / Bad 0 / 1.2 / 3.3 / 4.3 / 5.5 / 7	8
M-AMBI		Uses AMBI, S and H', combined with factor analysis and discriminant analysis.	
EcoQS:		High / Good / Moderate / Poor / Bad 1 / 0.82 / 0.61 / 0.4 / 0.2 / 0	9
BENTIX		= (6 × %GS + 2 × %GT)/100 where GS = GI + GII, GT = GIII + GIV + GV	
EcoQS:		High / Good / Moderate / Poor / Bad	14
Pollution classification:		Normal/pristine / Slightly / Moderately / Heavily / Azoic 6 / 4.5 / 3.5 / 2.5 / 2 / 0	14
MEDOCC		= [(0 × %GI + 2 × %GII + 4 × %GIII + 6 × %GIV)]/100 where GIV = GIV + GV	
EcoQS:		High / Good / Moderate / Poor / Bad 0 / 1.6 / 3.2 / 4.77 / 5.5 / 6	10
BOPA		= log (f_P / (f_A + 1) +1) where f_P is the opportunistic polychaete frequency (defined here as GIV + GV polychaetes); f_A is the Amphipoda frequency. 'Frequency' = ratio of total number of specified individuals out of total number of individuals in sample.	
EcoQS:		High / Good / Moderate / Poor / Bad 0 / 0.09 / 0.16 / 0.25 / 0.3 / -	15
ITI		= 100 – 33.33 × ((0 × n_1 + 1 × n_2 + 2 × n_3 + 3 × n_4) / (n_1 + n_2 + n_3 + n_4)) where n_1, n_2, n_3 and n_4 are the number of individuals in suspension detritus, interface detritus, surface deposit and subsurface deposit feeding groups, respectively.	
EcoQS:		Reference / Normal / Intermediate / Degraded 100 / 80 / 60 / 30 / 0	12
Biodiversity indicator:		High / Moderate / Reduced / Very low 100 / 50 / 25 / 5 / 0	16,17
BQI		= $(\sum_i (A_i/\text{tot}A \times ES50_{0.05i})) \times {}^{10}log(S + 1)$ Where $ES50_{0.05}$ is the species tolerance value, given here as the 5th percentile of the ES50 (expected number of species as per Hurlbert (1971)), scores for the given taxa. $A_i/\text{tot}A$ is the mean relative abundance of species *i* and S is the number of species for	18
Environmental status:		High / Good / Moderate / Poor / Bad 16 / 12 / 8 / 4 / 0	13

3.4 Evaluating index performance against ES

Relationships between environmental indicators and ES (as the explanatory variable) were described using polynomials of order 1, 2 and 3, to accommodate linear (i.e. order 1) and non-linear responses. The Akaike Information Criterion (AIC) statistic was used to determine the optimum model for both high and low flow data. The model with the lowest AIC was selected unless the difference was less than 2 (and therefore deemed essentially equivalent, Burnham & Anderson 2002), in which case the lowest order model with the best visual fitted shape was selected. If both high and low flow models were linear, they were compared using ANOVA with flow as a fixed factor, and using the 'slope.com()' function (test for common slope) in the 'smatr' library in R (R Development Core Team 2011).

The hypothesis that no significant difference existed between second and third order models (high versus low flow) was tested using a bootstrap procedure (Efron & Tibshirani 1993). Low flow and high flow datasets were combined assuming that they were from the same distribution, and then a bootstrap sample was drawn with replacement. Models were fitted to the bootstrap data and the difference of the coefficients (difBoot) was calculated and compared with the observed difference (difObs). This procedure was repeated 2000 times and the proportion of cases where difBoot was greater than difObs was used to estimate the level of significance (Efron and Tibshirani, 1993).

As ES values at high flow sites never exceeded 6, low flow data with ES scores greater than 6 were omitted from the comparative tests to ensure comparability in data ranges. If neither result was significant ($P<0.05$) then the data were combined and the model selection process was repeated. Where high and low flow models for a given response variable were best described using different order polynomials, then comparisons were made based only on the residual sum of squares, as the functional responses were considered to be different. The best fitting polynomials are displayed along with 95 % point wise confidence intervals.

3.4.1 Inter-relationships among variables

PCA (PRIMER v6) was use to examine inter-correlations among variables. Pearson correlations between the indicator variables and other farm-related variables, such as Depth, Current, Age, Distance and Feed, were illustrated with a vector plot overlaid on the two primary PCA axes (PCA1 and PCA2). Prior to PCA, individual variables were log transformed where appropriate (Distance, Feed, N and S^{2-}), missing values were replaced using the expected maximum likelihood algorithms (using the 'Missing' function in PRIMER v6) and all data were normalised.

3.4.2 Comparison of indicator classifications

Relationships between the different indicators, and assessment of their inferred EcoQS classifications (from Table 3-2) in relation to the stages along the enrichment gradient (as assessed by average BPJ), were examined with the aid of a nomogram. For the nomogram, boundary conditions between ES 1 – 7 were obtained for each of the indicator variables using the optimum models with ES as the explanatory (predictor) variable. The classifications in Table 3-2 represent previously established ranges for each indicator that were sourced from relevant literature. The various classifications were shaded consistently, with the darkest tone indicating the most impacted (e.g. enriched, worst, most anoxic) end of the spectrum.

3.5 Results

3.5.1 Versatility of benthic indicators in high and low flow environments

Two indicator variables exhibited statistically similar relationships with changes in ES for both high and low flow datasets combined; Redox (Figure 3-2) and the M-AMBI (Figure 3-3). Both of these involved first order polynomials, however, the relationship for the combined high and low flow regression was considerably more robust for the M-AMBI (R^2 = 0.819) than for redox (R^2 = 0.609, Table 3-3). Despite the reasonably good combined linear relationship with M-AMBI, there was still some undesirable variability, and therefore limited discriminatory power, at the upper end of the enrichment scale (ES 6 – 7). Hence, no one indicator performed well and comparably for both high and low flow datasets across the full enrichment

spectrum. Indicator variables AMBI, BENTIX, MEDOCC and BQI all had the same (second) order polynomials for the high and low flow data, and showed a similar response to enrichment up to ES 6. However, in each case one or more of their model coefficients was significantly different, hence the data could not be pooled (Appendix 3-B).

For low flow sites alone, ES was most strongly related to the BQI, with an adjusted R^2 of 0.909, followed by M-AMBI >> log(N) > d (Margalef's Richness) AMBI (Table 3-3). The BOPA and J' did a relatively poor job of describing ES at the low flow sites, with $R^2 < 0.6$. The usefulness of the relationships between log(N) and to a lesser degree, MEDOCC, were compromised by the fact that the data exhibited a parabolic (horseshoe-shaped) pattern, meaning one value of Y can have two possible values of X but at contrasting ends of the ES spectrum, Figures 3-2 and 3-3). The ITI, BENTIX and MEDOCC showed good responses up until ES4, as indicated by a strong slope and limited scatter about the mean, but the relationships in the range of ES4 to ES6 were less well defined, and beyond ES6 the responses were extremely variable. The AMBI also had a large amount of scatter in the data at ES > 5.5, which adversely influenced the regression by reducing (pulling down) the upper part of the curve/line. Better fits (where R^2 increased by >0.1) were obtained for %OM, J', AMBI, BENTIX, and MEDOCC when the data were restricted to only include results from ES1 – ES6. Indicators d, H', BQI and the M-AMBI appropriately indicated a highly impacted state beyond ES 5, but their change in slope (hence sensitivity) between ES5 and ES 7 was low.

For high flow sites alone, the measures that showed the greatest correspondence with ES were the indices MEDOCC, AMBI, M-AMBI, BENTIX, and BQI (in that order), with similar adjusted R^2 values of between 0.881 and 0.831 (Table 3-3 and Appendix 3-B). In all cases except the M-AMBI, the best-fit model was a second order polynomial; the M-AMBI was best described by a first order polynomial. The relationships between %OM and S, BOPA and Redox, and ES were relatively weak ($R^2 < 0.5$). Both %OM and S showed minimal response to increasing ES up until ES ≈ 4, whereas log(N) increased progressively through all stages of enrichment up until ES5.5 (the limit of the data, Figure 3-2).

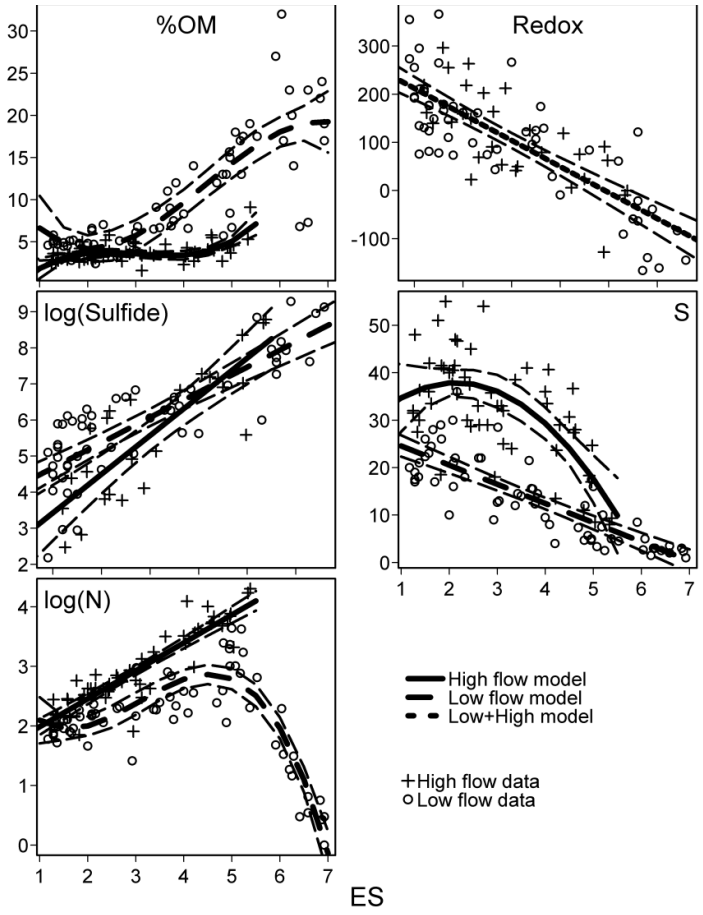


Figure 3-2: Scatter plots displaying optimum models (thick lines) with 95% confidence intervals (thin dashed lines) for each of the physico-chemical and biological indicators in response to Enrichment Stage (ES, as assessed by average BPJ). Thick solid and dashed lines indicate the best-fit models for high and low flow data, respectively, except for M-AMBI for which high and low flow data were pooled. Corresponding polynomials and model fit statistics provided in Appendix 3-B.

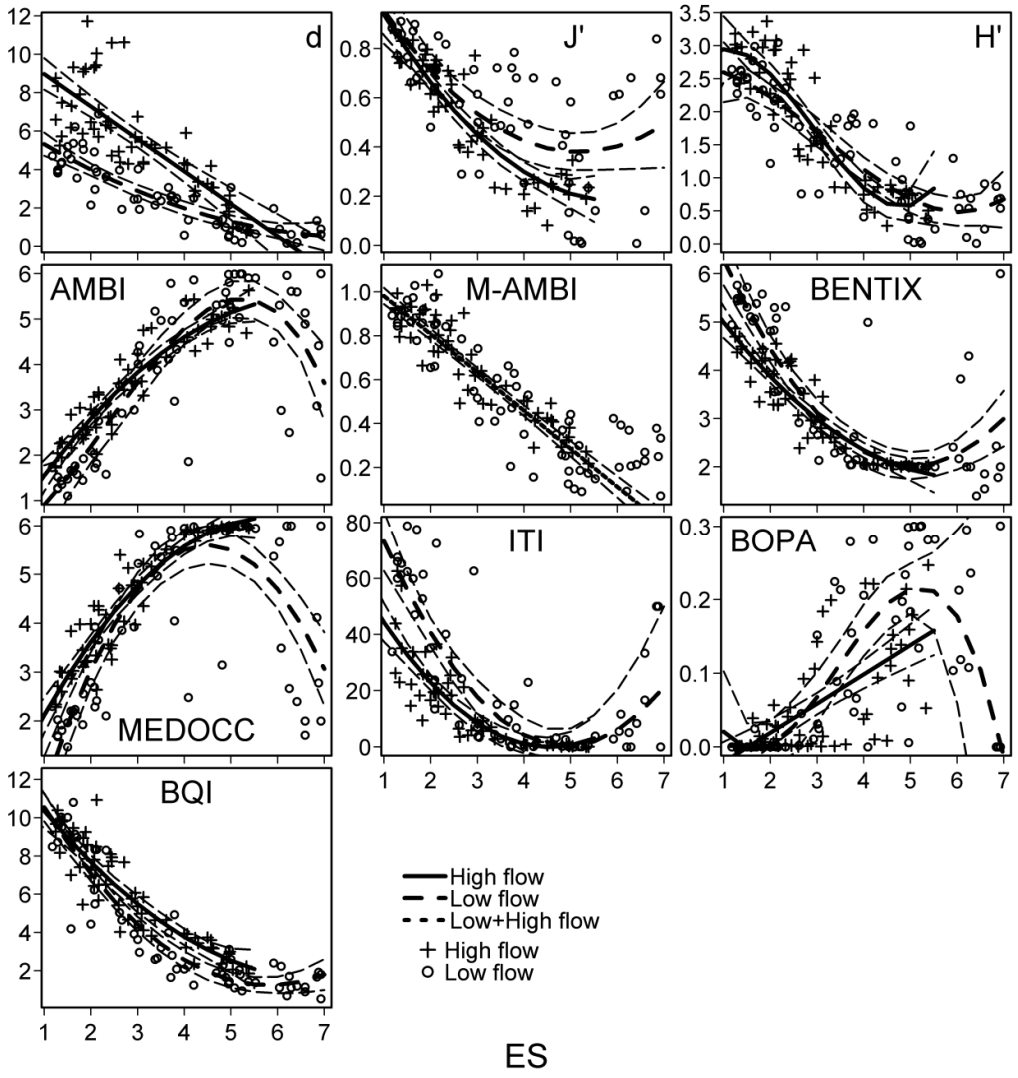


Figure 3-3: Scatter plots displaying optimum models (thick lines) with 95% confidence intervals (thin dashed lines) for the ten biological indicators, in response to Enrichment Stage (ES). Thick solid and dashed lines indicate the best-fit models for high and low flow data, respectively, except for M-AMBI for which high and low flow data were pooled. Corresponding polynomials and model fit statistics provided in Appendix 3-B.

Table 3-3: Rank orders for biotic indices according to associated adjusted R^2 values, ordered according to the low flow R^2 values. Overall rank determined from ordering low and high flow regressions in same series (inclusive of low regressions truncated to ES<6). Adjusted R^2 values also given for low flow data truncated to ES<6 for comparison. Top five ranks in each column shaded black, ranks 6-10 shaded grey, and 11-15 un-shaded. Dash (-) indicates that either the order of the polynomials were different for the HF and LF models, and/or that the models were of the same order, but were not significantly different. Refer to Appendix 3-B for full model statistics.

	Low Flow				**Low Flow ES<6**		**High Flow**				**Combined**	
Variable	Poly. order	Adj. R^2	Overall rank	Flow rank	Poly. order	Adj. R^2	Poly. order	Adj. R^2	Overall rank	Flow rank	Poly. order	Adj. R^2
BQI	2	0.9097	1	1	2	0.8602	2	0.8307	7	5	-	-
M-AMBI	3	0.8325	6	2	1	0.8058	1	0.8501	4	3	1	0.8195
Log(N)	3	0.744	11	3	1	0.413	1	0.824	10	8	-	-
d	2	0.7371	12	4	1	0.6824	1	0.6261	24	11	-	-
AMBI	3	0.732	13	5	2	0.8322	2	0.8774	3	2	-	-
H'	2	0.7212	14	6	1	0.6793	3	0.8292	8	6	-	-
BENTIX	2	0.7199	15	7	2	0.8408	2	0.8469	5	4	-	-
S	1	0.6937	17	8	1	0.5457	2	0.4386	28	14	-	-
ITI	2	0.6868	18	9	2	0.7368	2	0.7093	16	9	-	-
log(S^{2-})	1	0.6807	19	10	1	0.6218	1	0.6803	20	10	-	-
%OM	3	0.6712	21	11	2	0.7754	3	0.3518	30	15	-	-
MEDOCC	2	0.6492	22	12	2	0.8002	2	0.8818	2	1	-	-
Redox	1	0.6453	23	13	1	0.5648	1	0.4956	26	12	1	0.6098
BOPA	3	0.5479	25	14	1	0.5937	1	0.4433	27	13	-	-
J'	2	0.43	29	15	1	0.5623	2	0.8265	9	7	-	-

3.5.2 Relationships between indicators and comparison of ecological classifications

Results of the PCA largely reflected the findings of the univariate response models in Figures 3-2 & 3-3. The ES variable acted in a direction consistent with PC1 (horizontal on x-axis), hence any indicators oriented on that axis were both correlated to ES and not greatly influenced by high or low flow characteristics (Figure 3-4-A). Thus, BOPA, AMBI and to a lesser extent MEDOCC, were positively correlated with ES, whereas H', BQI and M-AMBI were strongly negative correlated with ES, along with Distance from Cages (Figure 3-4-A). MEDOCC and AMBI were also strongly negatively correlated with BENTIX, J' and ITI. S, log(N), d, and MEDOCC, were moderately influenced by flow characteristics; tending to be higher at high flow sites. %OM and to a lesser degree, ITI and J' were also influenced by currents speed, but tended to be higher at low flow sites. Very similar relationships were evident between these variables in the 2009 and 2010 dataset (Figure 3-4-B); M-AMBI was strongly negatively correlated with ES, whilst AMBI, BOPA and to a lesser degree MEDOCC, were positively related

to ES. S^{2-} and redox were strongly negatively related to each other and also reasonably well aligned with the ES axis. High flow samples were best distinguished from low flow samples by N and S, as well as by mud content (Mud) and %OM (both being typically higher at low flow sites).

The nomogram of classification schemes for each variable demonstrates inconsistencies among some of the indicators (Figure 3-5). Sediment chemistry indicators were largely unresponsive over the early stages of enrichment, with sediments classified as Oxic-A or 'natural' up to ~ES4 (Figure 3-5). Likewise, the percentage reduction in S and the BOPA index classified sediments as being of 'High' biodiversity and EcoQS (respectively) throughout the same early stages of enrichment, particularly at the high flow sites. In contrast, some of the diversity and biotic indices suggested that even the 'natural' (reference site) conditions observed in this study, were of moderate, reduced, or poor ecological quality (e.g. H', ITI and BQI) or generally less than pristine (d). Similarly, at the upper end of the enrichment spectrum (i.e. ES 5-7) a wide variety of environmental qualities were indicated. H', M-AMBI, BENTIX, ITI, BQI and MEDOCC all indicated that the lowest/ worst biodiversity or most degraded EcoQS had been achieved, while the BOPA, redox, and to a lesser extent, S^{2-}, indicated only moderately enriched conditions at ~ES6.

Irrespective of the scaling, several of the diversity measures and biotic indices (most notably, J', M-AMBI, BENTIX and MEDOCC), provided a response that suggested a predictable progression through the degenerative categories, in a manner consistent with the ES gradient (Figure 3-5). Classifications at the upper end of the enrichment spectrum were underestimated for several of the indicators, including J', AMBI, BENTIX, MEDOOC and the BOPA, due to the spurious EcoQS values that were produced when the macrofauna community was severely compromised, and S and N were accordingly very low.

The indicators showed quite individual response patterns to the two different flow environments. %OM, S and d were less responsive at the high flow sites than at the low flow sites (Figures 3-2 & 3-3). %OM doubled from ~3.3 % to ~6.6 % (i.e. ~100% increase) under highly enriched conditions at high flow sites whereas a five-fold increase (from ~4.2% to 19.3 %, or a 400% increase) was typical of the low flow sites under at the same stages of ES. However, once S was compromised at the high flow sites, it tended to reduce abruptly, and indicate highly impacted conditions earlier than for the low flow sites (Figure 3-5). S^{2-}, J', H',

BENTIX and ITI also tended to achieve the most impacted category for each at earlier stages on the ES scale at high flow sites. By contrast, AMBI, M-AMBI, BOPA and BQI all indicated slightly worse ecological statuses for low flow sites compared with high flow sites at equivalent enrichment levels greater than ES4.

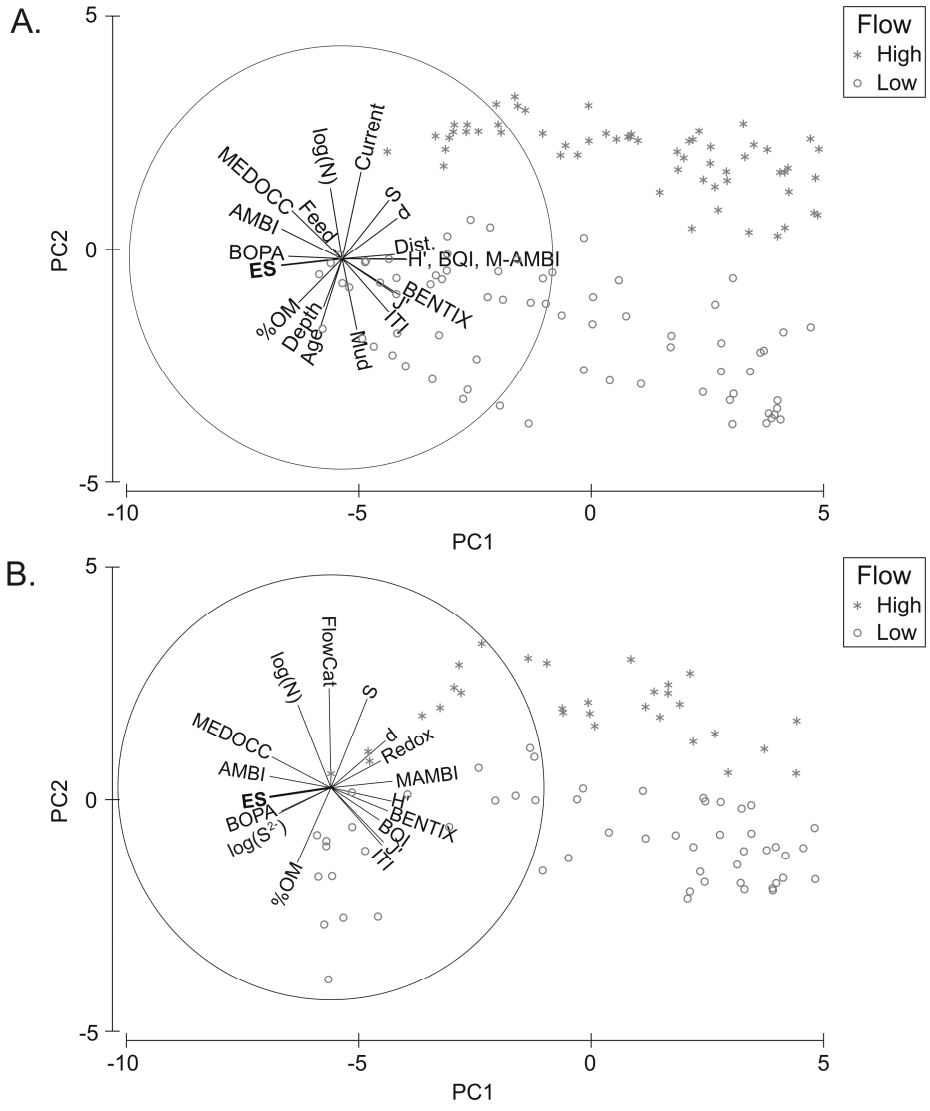
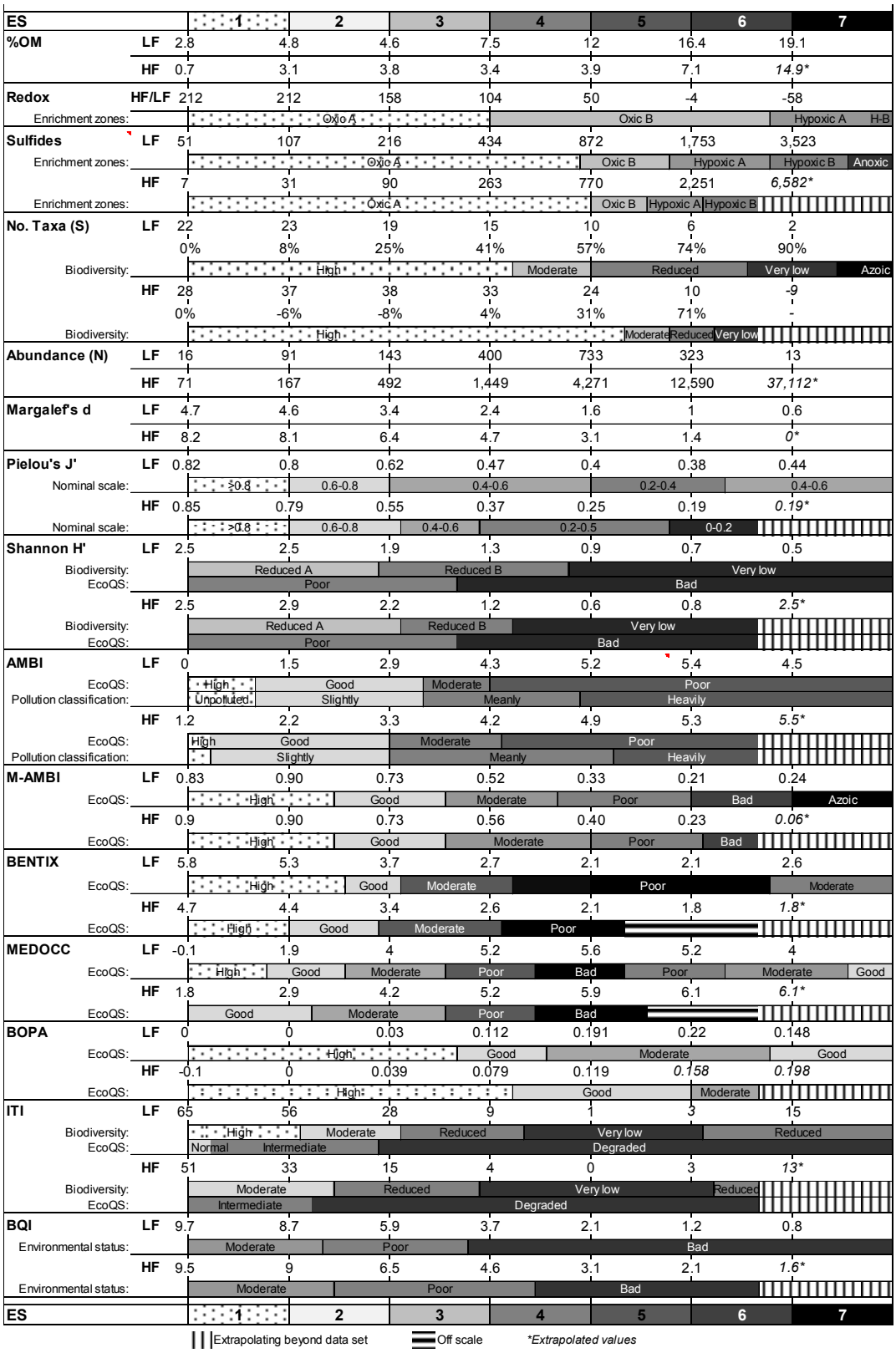


Figure 3-4: 2-dimensional PCA ordinations (based on Euclidean distances) of normalised farm and environmental indicator values (high and low flow data combined), overlaid with associated vector plots of Pearson correlations between variables. Plot A: primary dataset (i.e. all farms 2003-2009, excludes S2- and redox data); Plot B: secondary dataset (i.e. 2009 and 2010 inclusive of S2- and redox data). Cumulative variation explained by PC1 and PC2 = 69.0% (Plot A) and 79.7% (Plot B).

Figure 3-5 (overleaf): Nomogram comparing the classifications given by the results of the best-fit models in relation to published ecological classifications and quality standards. Refer to Table 3-2 for classifications schemes, boundary conditions and relevant reference sources. '*' and black vertical lines indicates estimates where the model has been used to extrapolate beyond the data and may be unreliable. Classifications are shaded consistently, with the darkest tone indicating the most impacted (e.g. enriched, worst, most anoxic) end of the spectrum. Shading with black horizontal lines indicates areas where the results are outside of the relevant scale.


ES 1 2 3 4 5 6 7
%OM LF 2.8 4.8 4.6 7.5 12 16.4 19.1
HF 0.7 3.1 3.8 3.4 3.9 7.1 14.9*
Redox HF/LF 212 212 158 104 50 -4 -58
Enrichment zones: Oxic A Oxic B Hypoxic A H-B
Sulfides LF 51 107 216 434 872 1,753 3,523
Enrichment zones: Oxic A Oxic B Hypoxic A Hypoxic B Anoxic
HF 7 31 90 263 770 2,251 6,582*
Enrichment zones: Oxic A Oxic B Hypoxic A Hypoxic B
No. Taxa (S) LF 22 23 19 15 10 6 2
0% 8% 25% 41% 57% 74% 90%
Biodiversity: High Moderate Reduced Very low Azoic
HF 28 37 38 33 24 10 -9
0% -6% -8% 4% 31% 71% -
Biodiversity: High Moderate Reduced Very low
Abundance (N) LF 16 91 143 400 733 323 13
HF 71 167 492 1,449 4,271 12,590 37,112*
Margalef's d LF 4.7 4.6 3.4 2.4 1.6 1 0.6
HF 8.2 8.1 6.4 4.7 3.1 1.4 0*
Pielou's J' LF 0.82 0.8 0.62 0.47 0.4 0.38 0.44
Nominal scale: >0.8 0.6-0.8 0.4-0.6 0.2-0.4 0.4-0.6
HF 0.85 0.79 0.55 0.37 0.25 0.19 0.19*
Nominal scale: >0.8 0.6-0.8 0.4-0.6 0.2-0.5 0-0.2
Shannon H' LF 2.5 2.5 1.9 1.3 0.9 0.7 0.5
Biodiversity: Reduced A Reduced B Very low
EcoQS: Poor Bad
HF 2.5 2.9 2.2 1.2 0.6 0.8 2.5*
Biodiversity: Reduced A Reduced B Very low
EcoQS: Poor Bad
AMBI LF 0 1.5 2.9 4.3 5.2 5.4 4.5
EcoQS: High Good Moderate Poor
Pollution classification: Unpolluted Slightly Meanly Heavily
HF 1.2 2.2 3.3 4.2 4.9 5.3 5.5*
EcoQS: High Good Moderate Poor
Pollution classification: Slightly Meanly Heavily
M-AMBI LF 0.83 0.90 0.73 0.52 0.33 0.21 0.24
EcoQS: High Good Moderate Poor Bad Azoic
HF 0.9 0.90 0.73 0.56 0.40 0.23 0.06*
EcoQS: High Good Moderate Poor Bad
BENTIX LF 5.8 5.3 3.7 2.7 2.1 2.1 2.6
EcoQS: High Good Moderate Poor Moderate
HF 4.7 4.4 3.4 2.6 2.1 1.8 1.8*
EcoQS: High Good Moderate Poor
MEDOCC LF -0.1 1.9 4 5.2 5.6 5.2 4
EcoQS: High Good Moderate Poor Bad Poor Moderate Good
HF 1.8 2.9 4.2 5.2 5.9 6.1 6.1*
EcoQS: Good Moderate Poor Bad
BOPA LF 0 0 0.03 0.112 0.191 0.22 0.148
EcoQS: High Good Moderate Good
HF -0.1 0 0.039 0.079 0.119 0.158 0.198
EcoQS: High Good Moderate
ITI LF 65 56 28 9 1 3 15
Biodiversity: High Moderate Reduced Very low Reduced
EcoQS: Normal Intermediate Degraded
HF 51 33 15 4 0 3 13*
Biodiversity: Moderate Reduced Very low Reduced
EcoQS: Intermediate Degraded
BQI LF 9.7 8.7 5.9 3.7 2.1 1.2 0.8
Environmental status: Moderate Poor Bad
HF 9.5 9 6.5 4.6 3.1 2.1 1.6*
Environmental status: Moderate Poor Bad
ES 1 2 3 4 5 6 7
Extrapolating beyond data set
Off scale
*Extrapolated values

3.6 Discussion

The objective of this study was to evaluate how well currently available indicators provided an ecologically relevant classification of both enrichment level and ambient environmental conditions, with a view to identifying metrics that could be widely applied. Ideally such indicators would need to show good discriminatory power across a full spectrum of benthic enrichment conditions, and under a range of regional environmental conditions (i.e. varying current flow regimes). Unfortunately, none of the metrics evaluated met these criteria. One of the main challenges appears to be the ability of the indices to reliably discern differences in the later stages of enrichment. In particular, distinguishing the situations where the macrofauna is still dominated by opportunists (and therefore still relatively abundant), but tending towards azoic. In the current study this part of the enrichment spectrum (ES values >5.5) was only apparent in the low flow dataset. Enrichment stages greater than 5.5 produced spurious responses for several indices (ITI, BOPA, MEDOCC, AMBI, BENTIX and J'), and relatively poor model fits. Regressions for most of these indices were substantially improved when ES > 6 data were excluded, with good predictive confidence being achieved between ES 1 – 6 (i.e. between un-enriched and post-peak abundance).

Low total abundance can adversely affect the performance of several indices (e.g., Borja & Muxika 2005, Dauvin & Ruellet 2007), and may underpin some of the issues with assessment above ES5 in the case of the low flow analyses, as abundance greatly declined in a manner consistent with the Pearson and Rosenberg (1978) enrichment model. However, ES 6 is an important stage on the enrichment spectrum and implicit in the environmental monitoring of finfish farms (Wilson et al. 2009), and consequently deficiencies in index performance in this region of the enrichment spectrum warrant further consideration. In some cases the problems can be associated with the particular way in which the index is characterised, for example in the case of BOPA the lack of fit at ES>5.5 was largely due to the presence of low numbers of amphipods (more specifically Melitidae and Haustoriidae) when opportunistic polychaetes were substantially diminished or absent. This caused a zero, or near zero, result indicative of an undisturbed environment, which is clearly erroneous as the conditions were assessed to be near-azoic, with only trace numbers (N = 1 – 14) of scavenging, and probably transient, surface dwelling macrofauna. It also has implications regarding regional endemism and the underlying assumption with this index that amphipods typify undisturbed environments, which is not

necessarily the case in our study region (Keeley et al. 2012b). Interestingly, when the BOPA was calculated using all opportunistic taxa (i.e. all EG IV and V), as opposed to just polychaetes, the relationship to ES for high flow sites was very good (R^2 = 0.926). This was mainly due to the inclusion of nematodes, which are a dominant part of the benthic fauna at the high flow sites; and appear to be important indicator taxa in this instance. Thus, indices that are based on a limited number of taxa are more prone to biases from endemism and are therefore unlikely to be suitable for broad geographical comparisons. Similarly, poor fits between ES and AMBI, BENTIX and MEDOCC were usually due to occasional individuals from EG's I - III influencing the result when overall abundances were very low. Although this problem has been acknowledged for the AMBI, the suggested criteria for the application of this index (>3 taxa and/ or >3 individuals, Borja & Muxika 2005) did not encompass all of the situations identified here. For example, some slightly more numerous samples from ES 6 type sediments were assessed to have an AMBI of 2-3, which indicates a 'Good' ecological quality standard.

Enrichment gradients at low flow sites were best described by BQI. This was largely attributable to the ability to obtain $ES50_{0.05}$ values for a high proportion of the taxa, and the fact that those values were derived from a larger, regionally specific database. BQI scores are also a function of S and moderated by an abundance factor (and half constant; Leonardsson et al. 2009), and as such index values tend to be appropriately suppressed when S and/ or N are low (i.e. ES>5.5). Fleischer et al. (2007) recommended replacing S with ES50 to overcome sampling effort biases, but this was not tested in the current study, because in aquaculture monitoring sampling effort does not generally vary, and this is unlikely to change in the near future. Similarly, the M-AMBI is a function of AMBI, H' and S, consequently the multivariate factor analysis approach had a positive influence on its ability to differentiate the highly enriched conditions that were encountered at the low flow sites. Incorporating species richness (S) into the calculations and being able to set site specific reference conditions also improved the versatility of the BQI and the M-AMBI, which performed well, with minimal spurious results for both high and low flow sites. However, between ES 5-7 the slopes of the regressions for these indices were negligible, revealing that they have a limited ability to clearly discriminate changes in ES within that range. The reason for this is that these stages are characterised by declining abundances of the same few resilient taxa, and therefore any change in the index score is almost entirely dependent on the influence of N and S in the calculation.

Despite having a reasonably tight relationship with ES, log(N) was deemed unsuitable for predictions at low flow sites because the parabolic shape of the polynomial means that a single N value can have two possible ES values – often at opposing ends of the enrichment gradient (Figures 3-3 & 3-5). However, N is still a valuable indicator because it is a simple intuitive measure that helps to identify the point of 'peak abundance' and post-peak declines (as alluded to above), and has meaning in the context of the Pearson Rosenberg model. Similarly, the number of taxa (S) at high flow sites displayed a parabolic response to ES, whereby an initial increase was followed by a pronounced decline at ES > 4. As a result, quantitative predictions of ES based on N and/ or S should always be validated by some other means.

The ability of the biotic indices to accommodate the extreme abundances that occurred under high flow conditions was evaluated by comparing the high flow model fits to the better-understood low flow enrichment responses. In general, index fits were better for high flow sites than for low flow sites, largely because the conditions associated with breakdowns in abundance/ diversity relationships found at ES > 6 were not encountered. When conditions > 6 were excluded from the low flow analysis, the fits for AMBI, BENTIX, MEDOCC and ITI were improved, and more comparable between high and low flow. The MEDOCC > AMBI > M-AMBI > BENTIX > BQI all did a good job of predicting enrichment stage at high flow sites, explaining 83 – 88 % of the overall variation, and were all strongly either positively or negatively correlated with ES. With the exception of the BQI, all of these indices are derived from the same base taxa classifications (EG's I-V). Therefore, contrary to the recommendations of Salas et al. (2006), classifying and accounting for opportunistic taxa (i.e. EG V) in this manner does appear to be a useful foundation for discerning organic enrichment. The comparable performance of these three indices also indicates that the level of EG differentiation used by the AMBI is unnecessary or partially redundant for this purpose. This finding is consistent with that of Simboura and Argyrou (2010) who also found good agreement between the BENTIX and MEDOCC, but proposed that the BENTIX was the more sensitive index due to the equal roles played by tolerant (EG III) and opportunistic (EG IV & V) taxa. In the present study, it is worth noting that the relatively limited response of S at the high flow sites is likely to have adversely influenced the discriminatory power of both the M-AMBI and BQI.

While many of the relationships between the indicators and ES were strong in terms of the residual sum of squares, there were significant inconsistencies between the indicators in

terms of the ecological quality or biodiversity statuses that were inferred. This was apparent throughout the enrichment spectrum, and depends on indicator type. Moderately impacted sediments (i.e., ES3-4) were recorded as healthy/ unimpacted according to chemical indicators, %OM, S (for high flow sites) and BOPA, while conversely, some diversity measures (d, H', BQI, ITI) indicated moderate impacts for sediments that were determined by average BPJ as being natural/ unimpacted. Abnormally low scores of d and H' are likely a result of small sample sizes (Sofia 2010) and the associated potential for under-sampling of rare species (MacArthur & Wilson 1967, in Hill 1973). However, the classification inconsistencies that were observed with some of the other indicators (especially BQI, BOPA, ITI, S^{2-} and redox) warrants further investigation, and reinforces the need for regional validation. At the upper end of the enrichment gradient, some indicators gave erroneously optimistic responses post-peak of opportunistic taxa. One such example was the BENTIX, which is known to have a tendency towards extreme values because it only recognises sensitive species (i.e., EG I) and opportunistic species of the first and second order (i.e., EG IV and V, Salas et al., 2006).

A final point is that contrasting responses at high and low flow sites were observed for some variables. In particular, the physico-chemical indicators and S, proved to be generally less sensitive over the early stages of enrichment at high flow sites (ES 1 – 4) compared with most infauna based indices. Hence, although some physico-chemical measures showed little or no response at high flow sites in the early stages of enrichment, significant macrofaunal changes occurred that were readily detected by indices such as the BQI, AMBI, M-AMBI and J'. Such results highlight the importance of including biological measures when assessing environmental quality.

3.7 Conclusion

An ideal benthic enrichment indicator would consistently discriminate the full enrichment gradient under a wide range of environmental conditions. None of the individual indicators that were evaluated in the contrasting flow environments described in this study fully met these criteria. However, the BQI > M-AMBI > AMBI > Log(N) > BENTIX all performed well, especially across ranges of moderate to high levels of enrichment. Of these, M-AMBI best catered for the different flow environments, while the BQI gave the least spurious

responses under highly enriched conditions. Most of the biological indicators showed limited ability to distinguish late stages of enrichment, when the macrofaunal population was on the decline. On the other hand, while physico-chemical measures provided a good level of discrimination over the later stages of enrichment, they were less sensitive than macrofauna for lower enrichment levels at high flow sites. Therefore, a useful subset for assessing enrichment status would comprise two of the best performing biotic indices that are based on alternative/ independent classification schemes (i.e. EG's and $ES50_{0.05}$), total abundance to aid in discerning PO, and a cheap and an easy-to-measure geochemical variable which responded consistently in the later stages of enrichment. Hence, the ultimate combination of variables for discerning enrichment gradients according to this study, would be: BQI + (M-AMBI / AMBI / BENTIX) + log(N) + Redox/S^{2-}.

In terms of identifying a single, universally applicable indicator, between-indicator inconsistency with respect to the inferred ecological status was a more significant issue than the was the ability of a single indicator to reliably discern conditions. Different indicators can have different biases with regard to site and/or region-specific characteristics. Consequently there is a very real risk of ecological status misclassification where a single index is used, particularly in the absence of regional validation. This has important ramifications for resource managers when attempting to identify environmental standards for broad regions or industry-wide applications. Although using a combination of indicators, as suggested above, is widely recommended (Borja & Muxika 2005, Muniz et al. 2005, Salas et al. 2006, Ranasinghe et al. 2009), the current study therefore also stresses the importance of selecting indicators that are regionally validated, and/or down-weighting the importance of any that are not. While using a combination of different indicators may be perceived to produce a 'safer' average result, where the calculations are based on poorly understood indicators the outcome may be misleading and the averaging effect has the potential to mask extreme conditions. Hence, there remains a clear need for expert judgement to select and appropriately weight indicator variables, to identify any spurious results, and to provide an integrated assessment of macrofaunal community condition - in particular to clarify when the community is "post-peak" abundance. This last point is particularly important given that most of the commonly-used indices we examined showed a tendency to error in the highest enrichment categories.

3.8 Acknowledgements

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3.9 Appendix

Appendix 3-A: $ES50_{0.05}$ scores and associated 'N' values that were determined for the 50 most abundant taxa and used to calculate BQI.

Taxa	General Group	Family	$ES50_{0.05}$	N	Rank
Cirratulidae	Polychaeta	Cirratulidae	6.62	1968	1
Heteromastus filiformis	Polychaeta	Capitellidae	6.00	1904	2
Nematoda	Nematoda		2.45	1845	3
Lumbrineridae	Polychaeta	Lumbrineridae	7.86	1840	4
Paraonidae	Polychaeta	Paraonidae	7.20	1787	5
Theora lubrica	Bivalvia	Semelidae	6.74	1757	6
Ostracoda	Ostracoda		7.62	1748	7
Cumacea	Cumacea		8.92	1729	8
Prionospio sp.	Polychaeta	Spionidae	5.45	1682	9
Arthritica bifurca	Bivalvia	Erycinidae	5.34	1460	10
Nemertea	Nemertea		6.31	1419	11
Cossura consimilis	Polychaeta	Cossuridae	8.65	1413	12
Sphaerosyllis sp.	Polychaeta	Syllidae	5.13	1333	13
Dorvilleidae	Polychaeta	Dorvilleidae	2.35	1316	14
Glyceridae	Polychaeta	Glyceridae	5.53	1283	15
Maldanidae	Polychaeta	Maldanidae	8.76	1255	16
Amphipoda	Amphipoda		4.16	1225	17
Armandia maculata	Polychaeta	Opheliidae	3.64	1108	18
Ophiuroidea	Ophiuroidea	Terrebellid	9.55	1101	19
Sigalionidae	Polychaeta	Sigalionidae	10.47	1101	20
Phoxocephalidae	Amphipoda		5.20	1086	21
Hesionidae	Polychaeta	Hesionidae	4.28	1075	22
Melitidae	Amphipoda		4.23	1074	23
Terebellidae	Polychaeta	Terebellidae	4.13	982	24
Capitella capitata	Polychaeta	Capitellidae	1.75	969	25
Tanaid sp.	Tanaidacea	Tanaidae	3.25	853	26
Syllidae	Polychaeta	Syllidae	3.93	822	27
Aglaophamus sp.	Polychaeta	Nephtyidae	8.96	752	28
Ennucula strangei	Bivalvia	Nuculidae	11.97	729	29
Nereidae	Polychaeta	Nereidae	3.64	699	30
Austrovenus stutchburyi	Bivalvia	Veneridae	4.63	695	31
Nucula gallinacea	Bivalvia	Nuculidae	4.40	639	32
Macrophthalmus hirtipes	Decapoda	Ocypodidae	5.00	639	33
Haustoriidae	Amphipoda		3.58	635	34
Spionidae	Polychaeta	Spionidae	4.06	630	35
Flabelligeridae	Polychaeta	Flabelligeridae	10.94	593	36
Polynoidae	Polychaeta	Polynoidae	5.46	561	37
Oligochaeta	Oligochaeta		4.46	534	38
Leptomya retiaria retiaria	Bivalvia	Semelidae	3.34	481	39
Phyllodocidae	Polychaeta	Phyllodocidae	3.61	477	40
Nucula hartvigiana	Bivalvia	Nuculidae	6.92	436	41
Notoacmea helmsi	Gastropoda	Lottiidae	4.65	418	42
Sipuncula	Sipuncula		7.15	406	43
Sabellidae	Polychaeta	Sabellidae	4.03	400	44
Prionospio multicristata	Polychaeta	Spionidae	3.79	391	45
Anthuridea	Isopoda	Anthuridea	9.99	371	46
Macomona liliana	Bivalvia	Tellinidae	6.62	367	47
Terebellides stroemi	Polychaeta	Trichobranchidae	11.08	363	48
Asellota	Isopoda		6.04	360	49
Nemocardium pulchellum	Bivalvia	Cardiidae	10.97	350	50

Appendix 3-B: Optimum models and associated fit statistics for the biological variables and indices as predicted by each Enrichment Stage (ES). Diff. Sig. = the significance of the difference between the high and low flow polynomials, where: • <0.1, * <0.05, ** <0.01, ** <0.001. 'Int.' = intercept. First order polynomials are tested using a single factor ANOVA (Pr(>F)) and the Bartlett-corrected likelihood ratio stat testing for common slope (P-value given). Second and third order polynomials are compared using a non-parametric bootstrapping procedure which provides probabilities for the difference between each of the factors. High and low flow data with different order polynomials were considered to have different X-Y relationships and therefore no tests were applied. If no significant differences were observed the high and low flow data were combined to produce a single polynomial.

Variable	Flow Cat.	Order poly.	ES^3	ES^2	ES	Int.	RSE	df	Mult. R^2	Adj. R^2	p-value
%OM	LF	3	-0.271	3.625	-11.408	14.6775	3.926	61	0.6867	0.6712	2.239e-15
	HF	3	0.309	-2.817	8.103	-3.737	0.9435	52	0.3872	0.3518	1.104e-05
	Diff. Sig.	P-value	0.060·	0.095·	0.090·	0.095·					
Redox	LF	1			-54.960	263.546	74.14	52	0.6519	0.6453	1.634e-13
	HF	1			-52.262	270.221	67.35	28	0.513	0.4956	8.54e-06
	Diff. Sig.	P-value			0.657	0.3677					
	Combined(ES<6)	1			–54.15	266.49	71.34	82	0.6145	0.6098	<2.2e-16
S^{2-}	LF	3	124.5	-989.3	2551.5	-1668.8	1390	50	0.6536	0.6328	1.448e-11
	HF	3	327.0	-2658.9	68.152	-5197.2	928.1	21	76556	0.7321	8.13e-07
	Diff. Sig.	P-value	0.1540	0.1965	0.1615	0.0925·					
log(S^{2-})	LF	1			0.698	3.977	0.8746	52	0.6868	0.6807	1.029e-14
	HF	1			1.072	2.354	1.021	23	0.6936	0.6803	2.402e-07
	Diff. Sig.	P-value			0.0401*	0.0036**					
S	LF	1			–4.037	28.616	4.913	63	0.6985	0.6937	2.2e-16
	HF	2		–2.478	10.606	26.560	8.271	53	0.459	0.4386	8.505e-08
log(N)	LF	3	-0.072	0.670	–1.598	3.095	0.3988	61	0.756	0.744	<2.2e-16
	HF	1			0.469	1.517	0.2698	54	0.828	0.824	<2.2e-16
d	LF	2		0.105	–1.638	6.855	0.8844	57	0.746	0.7371	<2.2e-16
	HF	1			–1.690	10.666	1.624	54	0.6329	0.6261	2.395e-13
J'	LF	2		0.032	–0.341	1.262	0.1992	56	0.4496	0.43	5.475e-08
	HF	2		0.030	–0.367	1.274	0.0974	53	0.8328	0.8265	<2.2e-16
	Diff. Sig.	P-value		0.1185	0.0810·	0.125					
H'	LF	2		0.060	–0.885	3.685	0.4708	62	0.73	0.7212	<2.2e-16
	HF	3	0.090	–0.795	1.375	2.278	0.394	52	0.8386	0.8292	<2.2e-16

Appendix 3-B continued.

Variable	Flow Cat.	Order poly.	ES^3	ES^2	ES	Int.	RSE	df	Mult. R^2	Adj. R^2	p-value
AMBI	LF	3	-0.058	0.413	0.450	0.099	0.8976	61	0.7446	0.732	<2.2e-16
	LF(<ES6)	2		–0.177	2.274	-1.539	0.7192	50	0.8387	0.8322	<2.2e-16
	HF	2		–0.119	1.615	0.055	0.3976	53	0.8819	0.8774	<2.2e-16
	Diff. Sig.	P value		0.5395	0.298	0.058·					
M-AMBI	LF	3	0.010	-0.097	0.100	0.937	0.122	61	0.8403	0.8325	<2.2e-16
	LF(<ES6)	1			-0.181	1.171	0.1291	51	0.8095	0.8058	<2.2e-16
	HF	1			-0.167	1.149	0.0852	54	0.8529	0.8501	<2.2e-16
	Diff. Sig.	P value			0.3308	0.1979					
	Combined(ES<6)	1			–0.174	1.155	0.109	114	0.821	0.8195	<2.2e-16
BENTIX	LF	2		0.258	–2.617	8.643	0.7872	62	0.7287	0.7199	< 2.2e-16
	LF<ES6	2		0.270	–2.694	8.746	0.5909	50	0.847	0.8408	< 2.2e-16
	HF	2		0.119	–1.480	6.352	0.3804	53	0.8525	0.8469	< 2.2e-16
	Diff. Sig.	P-value		0.068·	0.022*	0.001**					
MEDOCC	LF	2		–0.407	3.668	-2.657	1.016	62	0.6602	0.6492	2.943e-15
	LF<ES6	2		–0.271	2.832	-1.609	0.7676	50	0.8079	0.8002	<2.2e-16
	HF	2		–0.171	2.010	0.286	0.4193	53	0.8861	0.8818	<2.2e-16
	Diff. Sig.	P-value		0.3005	0.197	0.041*					
ITI	LF	2		5.066	–48.810	117.939	14.29	62	0.6966	0.6868	<2.2e-16
	LF<ES6	2		4.779	–46.904	115.426	13.7	50	0.7469	0.7368	1.204e-15
	HF	2		3.495	–31.897	72.705	7.955	53	0.7199	0.7093	2.258e-15
	Diff. Sig.	P-value		0.3675	0.135	0.0115*					
BOPA	LF	3	-0.008	0.085	-0.207	0.143	0.069	58	0.5701	0.5479	1.096e-10
	LF<ES6	1			0.060	-0.099	0.072	51	0.6015	0.5937	9.15e-12
	HF	1			0.039	-0.060	0.054	54	0.4534	0.4433	1.298e-08
	Diff. Sig.	P-value			0.018*	0.120					
BQI	LF	2		0.309	-4.059	14.119	0.970	62	0.9125	0.9097	<2.2e-16
	HF	2		0.230	-3.355	13.513	1.052	53	0.8381	0.832	<2.2e-16
	Diff. Sig.	P-value		<0.078·	0.0065**	0.0025**					

CHAPTER 4

NOVEL OBSERVATIONS OF BENTHIC ENRICHMENT IN CONTRASTING FLOW REGIMES WITH IMPLICATIONS FOR MARINE FARM MONITORING AND MANAGEMENT

Preface:

This chapter builds on the chapters 2 and 3 by more closely examining the relationships among and between biological and physico-chemical indicators (e.g. total free sulphide), and compare species richness (S), abundance (N) biomass (B) and trends under different flow regimes against the responses that characterise a classic conceptual model for organic enrichment developed by Pearson & Rosenberg model (1979). The findings and their relevance to current understanding of successional responses are reviewed, and some strengths and limitations of different environmental indicators for monitoring are identified.

This work has been published in a refereed journal and has been adjusted to a standard format for the thesis, and as such there may be minor differences in the text, figures and tables compared with the published version. The citation for the original publication is:

Keeley N, Forrest B, MacLeod C 2013. Novel observations of benthic enrichment in contrasting flow regimes with implications marine farm management. Mar. Pollut. Bull. 66, 105-116.

4.1 Abstract

We examine macrofaunal and physico-chemical responses to organic enrichment beneath salmon farms in contrasting flow environments, and reveal pronounced flow-related differences in the magnitude and spatial extent of effects. Total macrofaunal abundances at high flow sites were nearly an order of magnitude greater than at comparable low flow sites, representing a significant benthic biomass. These very high abundances occurred in conjunction with moderate-to-high species richness, and were evident in the absence of appreciable organic matter accumulation. Biological responses to increasing sulfide were variable; however a significant biological threshold was evident at 1500 µM. Macrofaunal responses at high flow sites differed substantially from the Pearson-Rosenberg model. The atypical ecological conditions were attributed to i) minimal accumulation of fine sediments, ii) maintenance of aerobic conditions in near-surface sediments, and iii) an abundant food supply. Thus, enhanced resilience to organic waste at well-flushed sites appears related to both biological and physical processes.

4.2 Introduction

Numerous studies have used environmental indicators to characterise benthic soft-sediment enrichment and disturbance gradients associated with marine point source discharges, such as ocean outfalls (e.g., Cardell et al. 1999), terrestrial inputs via rivers (e.g., Hermand et al. 2008, Labrune et al. 2012), oil fields (e.g., Olsgard et al. 1997) and aquaculture (e.g., Kalantzi & Karakassis 2006). An understanding of how environmental indicators relate to each other, change in response to increasing enrichment, and compare in different soft-sediment habitats is critical to interpreting these assessments (Keeley et al. 2012a). Biotic indices, in-particular, are increasingly used to guide assessments of environmental quality status (Llanso & Dauer 2002, Ranasinghe et al. 2007, Borja et al. 2009b, Dauvin et al. 2012); but the performance of such indices assumes comparable biological responses across different environments.

Pearson and Rosenberg (1978) provided a comprehensive assessment of benthic enrichment responses for soft-sediment macrofauna, which has become the foundation for many biotic indices, and the paradigm against which subsequent studies have been compared. An important contribution of the Pearson and Rosenberg study was the definition of species/ abundance/ biomass (hereafter referred to as 'SNB') curves characterising macrofaunal responses to organic inputs (often termed the Pearson-Rosenberg Model, or 'PRM'). Although the PRM has been shown to be widely applicable (Heip 1995), significant deviations have been identified under certain conditions. For example, Maurer et al. (1993) identified major departures from the model in terms of how SNB curves responded in high energy/ erosional habitats. In that instance, unusually sharp declines in SNB were observed toward azoic conditions and a proliferation of opportunistic species did not necessarily preclude rare species. Deviations from the model were also identified by Brooks et al. (2004), who described a site that appeared to lack the typical proliferation of opportunists under highly enriched conditions.

Sea-cage fish farms provide excellent case study systems for further evaluating enrichment effects and the general applicability of the PRM, as deposition of particulate organic matter in the form of faeces and waste feed can lead to pronounced gradients in benthic responses across small spatial scales. Typically, near-azoic conditions beneath fish farms progressively decrease in impact with distance from the cages, and natural conditions

are usually achieved within 100-200 meters (Brooks et al. 2002, Forrest et al. 2007, Giles 2008). The severity and spatial extent of effects is thought to be strongly influenced by current speed, whereby stronger currents aid dispersal, limit settlement of organic rich biodeposits (Cromey et al. 2002a, Giles et al. 2009) and promote oxygen flux to the sediments (Findlay & Watling 1997). Current speed also strongly influences abiotic properties, such as sediment grain size and compaction, which in turn, can also influence benthic biodiversity (McArthur et al. 2010).

Deep sites with strong water current flows are generally perceived to be relatively resilient to organic discharges (Frid & Mercer 1989, Hartstein & Rowden 2004, Borja et al. 2009b), although given sufficient organic inputs, the macrofauna beneath fish cages in high energy environments can nonetheless become highly modified (e.g., Keeley (e.g., Macleod et al. 2007, Keeley et al. 2012a). However, in some instances effects on the macrofauna at high flow sites may be poorly reflected by commonly used physico-chemical indicators, such as total organic matter (Aguado-Gimenez & Garcia-Garcia 2004, Aguado-Gimenez et al. 2007, Keeley et al. 2012a). Hence, water flow may not only influence macrofaunal SNB responses, but also the relative enrichment responses of macrofaunal versus physico-chemical indicators. Such possibilities have important ramifications for the application of established environmental indicators and biotic indices, and the extent to which they can be used to make inferences regarding ecological quality status (Aguado-Gimenez et al. 2007, Keeley et al. 2012a). For example, some biogeochemical parameters (total free sulfides and redox) are increasingly being promoted as key indicators to classify benthic enrichment gradients associated with fin-fish farms; most recently as a component of the World Wildlife Fund's global aquaculture standards (WWF 2012). While such approaches are relatively inexpensive and have appeal for their simplicity, it is important that these and other physico-chemical indicators accurately reflect biological responses. Unfortunately, this is difficult to gauge from the existing literature, as very few studies have compared the responses of a common suite of indicators across different flow regimes.

In this paper we extend the work of Keeley et al. (2012a), which identified some flow-specific and regional inconsistencies with a range of benthic environmental indicators, by more closely examining macrofaunal responses to enrichment. In particular, we examine the relationships among and between biological and physico-chemical indicators (e.g. total free sulfide), and compare SNB trends under different flow regimes against the classical responses

that characterise the PRM. We then review these findings and their relevance to our current understanding of successional responses along enrichment gradients in different flow environments, identifying the strengths and limitations of different environmental indicators for monitoring. Finally, we consider the implications of our findings for site selection in the context of sea-cage fish farming, and for subsequent assessment and monitoring of benthic effects.

4.3 Methods

4.3.1 The study sites and dataset

The data used in this assessment were extracted from a 14 year annual monitoring data set from six study sites, comprising salmon farms aged between 1 and 26 years (Farms A-F, Table 4-1), located within the Marlborough Sounds, New Zealand (Figure 4-1). All of the farms were fixed in position (with only minor adjustments) and operated relatively consistently throughout, with the exception of Farm-D, which was retired in 2001 and reinstated to full capacity in late 2008. The farms were situated in water depths of 27 - 40 m, and grouped according to their hydrodynamic properties; two of the farms (Farms E and F) had considerably greater current velocities (>15 $cm \cdot s^{-1}$, average at ~20 m depth) than the other four (<9 $cm \cdot s^{-1}$), and were designated as "high flow" and "low flow" groups, respectively. This *a-priori* grouping is based on the critical resuspension velocity threshold for farm-derived organic particulates of 9.5 $cm \cdot s^{-1}$ recommended by Cromey et al. (2002b) for use in depositional models. This threshold might therefore be expected to have an important bearing on the severity and spatial scale of benthic enrichment effects. The water current data defining the high and low flow regimes were obtained from 28-40 day current meter deployments at each site (SonTek™ 1 MHz Acoustic Doppler Profiler), which recorded current speeds averaged over 3 minutes at intervals of 15, 30 or 45 minutes. Stations for sampling sediment macrofauna and physico-chemical properties at each site included two beneath cage stations, two or three stations at increasing distances away from the cages (out to 250 m) and a reference station (>1 km away, Table 4-1). All of the sampling stations were situated over unconsolidated sediments, with low flow sites tending to sandy-mud, and high flow sites tending to muddy-sand according to the standard sediment textural classifications of Folk (1954) (Table 4-1).

Analyses were conducted on three subsets of the data, as not all parameters were measured consistently throughout the entire sampling period. The first dataset (Dataset 1) combined the information from all the sites over 17 different surveys spanning nine years (2001-2009). Dataset 1 included feed usage (Feed, total metric tonnes for 6 months prior to sampling) and covered a range of feed input levels (1640-4120 tonnes yr^{-1}) to represent potential extremes in enrichment levels. The farm information also included farm age at sampling (Age, years), and average current speed at ~20 m water depth (Current, cm s^{-1}). The information specific to each sample station included: water depth (Depth, m), site distance from farm (Distance, m), sediment grain size distribution (presented as %Mud), percent organic matter (%OM, measured as % ash free dry weight w/w; Luczak et al. 1997), and a detailed breakdown of the infaunal community structure. All sediment sampling was conducted using a boat-operated Van-Veen grab, with macrofauna collected by sub-sampling with a 13 cm diameter core (sample size: 0.0132 m^2, by 10 cm deep) and sieving to 0.5 mm. Macrofauna were sorted and enumerated to the lowest practicable level and their abundances (hereafter denoted N) recorded. We use 'N*' in places to denote total abundance exclusive of opportunistic taxa; defined in this instance as those species previously classified as first-order opportunists (i.e. Eco-Group V) according to Borja et al. (2000). Sediment grain size and %OM measures were based on sub-samples taken from the grab with a 5.5 cm diameter Perspex core, from which the surface 30 mm was retained for later analysis. Qualitative information was also obtained in the field at each sampling site of sediment odour (H_2S, Odour), bacteria mat coverage (*Beggiatoa*) and sediment out-gassing using pre-specified categories (Keeley et al. 2012b). Results from Dataset 1 were analysed using average values from duplicate or triplicate samples.

The second data set (Dataset 2) comprised environmental information from the same sites over the years 2009 - 2011 and included the same variables as in Dataset 1, with the addition of total free sulfide (TFS, µM) and redox potential (Eh_{NEH}, mV) (Table 4-1), and was analysed at the replicate level. Redox was measured directly from the grab (at 1 cm depth) using a Thermo Scientific combination Redox/ORP electrode. TFS was sampled with a cut-off 5-cc plastic syringe driven vertically into the surface sediments (0-4.5 cm depth interval), and the TFS contents were extracted and quantified following the methods of (Wildish et al. 1999). The third dataset comprised a detailed gravimetric analysis of macrofauna collected in May and November 2011 from all sample sites (cage through to reference) at each of the six farms (Table 4-1). In this instance, after taxonomic analysis, the dry weight of the whole macrofaunal

sample from each site was obtained by drying the samples on pre-weighed GFC filters (60 °C for 24 hrs), and then re-weighing on a digital balance (to 4 d.p.). For samples that had exceptionally high densities of nematode and capitellid worms, total dry weight estimates were made from sub-samples. Individual nematodes and capitellids were also separated, counted and weighed from a cross-section of samples, to obtain estimates of their average biomasses.

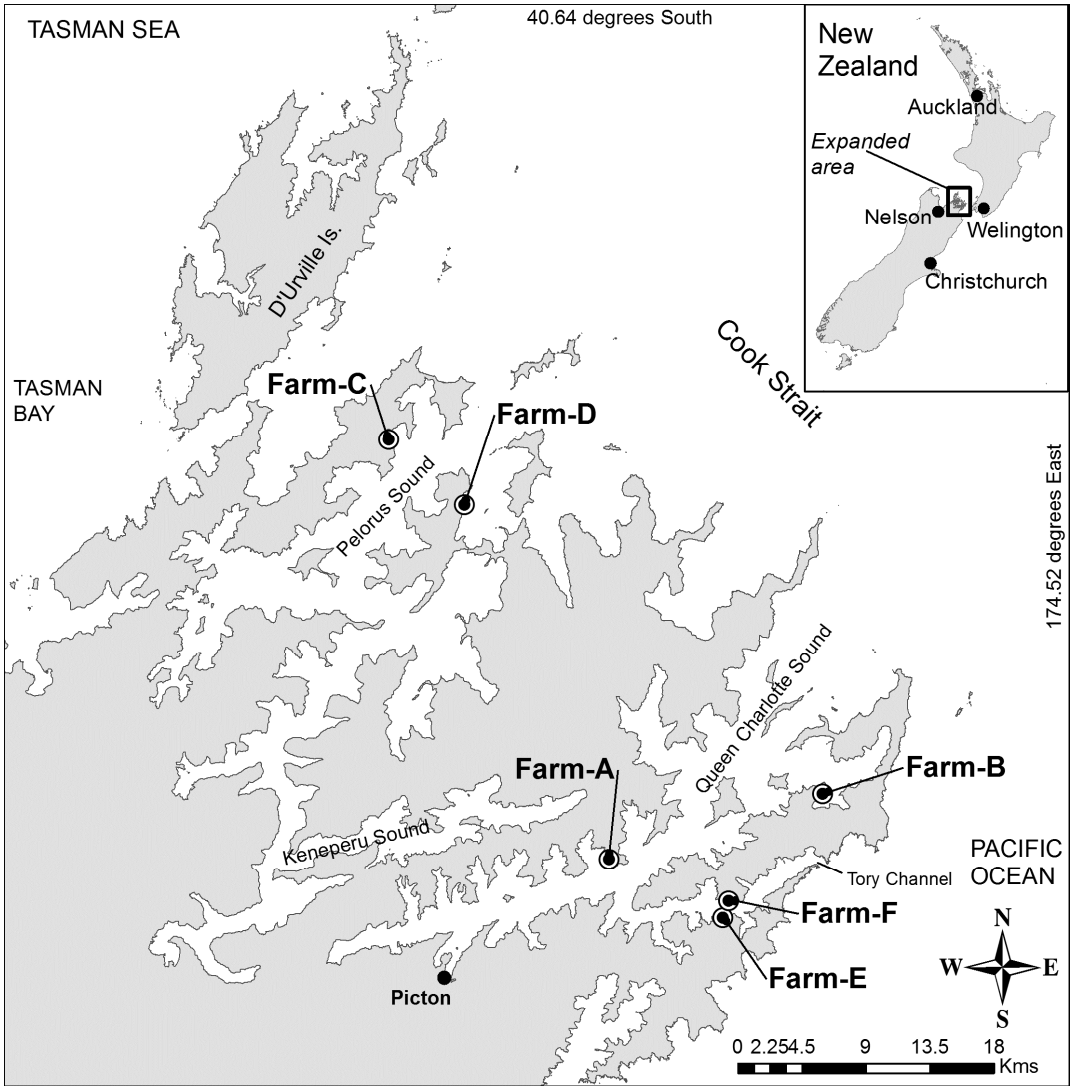

Figure 4-1: Location of the six salmon farm study sites, Marlborough Sounds, New Zealand.

Table 4-1: Summary of farm attributes for the six study sites.

		'Low flow' farms				'High flow' farms	
Farm attributes	**Units**	**Farm-A**	**Farm-B**	**Farm-C**	**Farm-D**	**Farm-E**	**Farm-F**
Farm established:	Year	1985	1989	1989	1994	1988	2007
Year of surveys:							
Dataset 1	20-	'04,'06,'07	'03,'05,'08,'09	'03,'05,'07,'09	'01	'03,'05,'08	'08,'09
Dataset 2	20-	'09,'10,'11	'09,'10,'11	'09,'10	'09,'10,'11	'09,'10,'11	'09,'10,'11
Dataset 3	20-	'11	'11	'11	'11	'11	'11
Site depths	m	34-35	37-39	28-30m	34-35	27-31	30-40
Sediment mud content	%(range)	80 (69-84)	55 (34-73)	78 (69-85)	84 (82-86)	28 (21-38)	32 (29-37)
Mean current speed							
Mid-water	cm s^{-1}	3.7 (17.5)	6 (34.6)	8.2 (29.9)	3.0 (10.1)	14.4 (53.8)	19.9(117)
Near-bottom	cm s^{-1}	3.5	3.5	8.1	3.2	15.4	19.0
Range of feed inputs	mt yr^{-1}	2510-3289	1640-2239	2171-3918	100-2264	2104-4120	2631-3526
Sampling stations (Dist. from cages)	m	0(×2),50, 150,250,Ref	0(×2),50, 150,250,Ref	0(×2),50(×2), 100,150,Ref	0(×2),25, 50,75,100, 150,Ref	0(×2),50, 200, Ref	0(×2),50(×2), 100(×2),150(×2), 200(×2),250(×2), Ref

4.3.2 Data analysis & characterisation

Overall trends in biological and physico-chemical parameters are shown graphically for high and low flow sites in relation to distance from fish farm cages. Shannon diversity (H') and the AMBI (AZTI's Marine Biotic Index, Borja et al. 2000) were calculated from the biological dataset. An additional continuous variable, "Enrichment Stage" (ES), was derived from the combined biological and physico-chemical parameters by a best professional judgement method described in Keeley et al. (2012b). This approach enabled each sampling station to be classified into one of seven ES categories ranging from unimpacted natural conditions (ES 1) to extremely enriched azoic conditions (ES 7), with ES 5 representing the classical abundance "peak of opportunists" described by the PRM (Table 4-2).

To assess compositional changes in the benthic assemblages, macrofaunal data were 4^{th} root transformed and a resemblance matrix was created using S17 Bray-Curtis dissimilarities, with a dummy variable of 1 added to avoid over-dispersion from samples with zero, or near-zero, abundance. Differences were displayed using 2-dimensional multidimensional scaling (MDS, PRIMER v6 Clarke 2006) plots. Samples were coded according to ES (1-7) and flow regime (low/high), and a second MDS bubble plot produced to graphically illustrate site distances from fish farm cages. The taxa that best characterised the high and low flow

samples under natural conditions (ES 1) and highly enriched (peak abundance) conditions (ES 5) were obtained using SIMPER analysis, with a 60% cut off for low contributing taxa.

A resemblance matrix of the environmental data was created using D1 Euclidean distance. The physical variables included: 'Distance', 'Feed', 'Age', 'Current', 'Depth' and '%Mud'. The physical attributes that best explained the differences between macrofaunal samples were compared by Spearman rank correlation using the BIOENV method in the BEST procedure (PRIMER v6). Sample variables were specific to each sampling event (Year × Farm × Sample site), with the exception of current speed, which was farm-specific, i.e. the same current velocities were used for all sites at a given farm. The assumption was made that hydrodynamic variation among stations within 'farms' was negligible (i.e. significantly less than differences between farms).

Inter-relationships among variables were assessed, including specific evaluation of correlations with TFS values from Dataset 2. This aspect included graphical comparison with established TFS relationships derived for northern hemisphere sites and described by Hargrave et al. (2008, 2010). Changes in total macrofaunal abundance (N), number of taxa (S) and total biomass (B) were characterised in response to the ES variable using quantile regression splines (Koenker et al. 1994, Koenker 2007). The 50^{th} and 95^{th} percentiles are displayed, which represent the value below which 50% and 95% of the data fall (respectively). Optimum models were selected, fitted and displayed using the methods proposed by Anderson (2008a) and later adapted by Keeley et al. (2012b) for characterising enrichment gradients. The ES score at which each of the variables peaked (i.e. achieved a maximum, or 'X-optimum') and the corresponding size of that peak ('Y-max') were similarly determined. For each of the three macrofaunal variables, the ES response curves were graphically compared with the stylised response to increasing organic enrichment described by the PRM.

Table 4-2: Narrative criteria describing seven enrichment stages, used by experts for best professional judgement (BPJ) assessments. Modified from Macleod and Forbes (2004) and Pearson and Rosenberg (1978).

ES	General description	Environmental characteristics
1	Natural/pristine conditions	Environmental variables comparable to unpolluted/ un-enriched pristine reference site.
2	Minor enrichment: Low level enrichment. Can occur naturally or from other diffuse anthropogenic sources. 'Enhanced zone'	Richness usually greater than for reference conditions. Zone of 'enhancement' – minor increases in abundance possible. Mainly compositional change. Sediment chemistry unaffected or with only very minor effects.
3	Moderate enrichment: Clearly enriched and impacted. Significant community change has occurred.	Notable abundance increase, richness and diversity usually lower than reference. Opportunistic species (i.e. capitellids) begin to dominate.
4	Major enrichment 1: Transitional stage between moderate effects and peak macrofauna abundance. Major community change.	Diversity further reduced, abundances usually quite high, but clearly sub-peak. Opportunistic species begin to dominate, but other taxa may still persist. Major sediment chemistry changes.
5	Major enrichment 2: Highly enriched. State of peak macrofauna abundance.	Very high numbers of one of two opportunistic species (i.e. capitellids, nematodes). Richness very low. Major sediment chemistry changes. Bacteria mat (Beggiatoa) usually evident. H2S out-gassing on disturbance.
6	Major enrichment 3: Transitional stage between peak and azoic.	Transitional stage between peak and azoic. Richness & diversity very low. Abundances of opportunistic species severely reduced from peak, but not azoic. Total abundance low but can be comparable to reference. %OM can be very high.
7	Severe enrichment: Azoic/abiotic; sediments no longer capable of supporting macrofauna. Organics accumulating.	None, or only trace numbers of macrofauna remain. Some samples with no taxa. Spontaneous out-gassing; Beggiatoa usually present but can be suppressed. %OM can be very high.

4.4 Results

4.4.1 Flow-specific responses to enrichment

From the long-term study (Dataset 1 & 2), un-enriched reference sediments (>1km from fish cages) from the two high flow sites (i.e. >15 cm s^{-1}, Farm-D and -E) had a coarser grain size (less mud and more sand, Table 4-1) and a lower sediment organic content (mean 3.2 %, range 2.5-4.2 %OM) than the low flow sites (typically ~5 %OM, Figure 4-2). Macrofaunal communities at reference stations were more diverse and abundant at the high flow stations, with approximately twice the number of taxa (S) and 2-3 times the total infaunal abundance (Figure 4-3).

Beneath the cages at the high flow farms (E & F), %OM was only slightly elevated (~1 - 4 %), whereas the low flow sites often had %OM levels of 10 – 20% (e.g. Figure 4-2). Average total abundances (N) beneath the cages at the high flow sites peaked at 39,877 (SE=11,259, n=3) individuals per core (Farm E, 2011), compared to ~4,398 (SE=1,286, n=3) individuals per core (Farm-A, 2011) for the low flow farms. The macrofaunal community found directly beneath the low flows sites was generally impoverished (near azoic), with S (1-3 taxa) reduced by >90 % compared with corresponding reference sites (Figure 4-3). By contrast, S at the high flow cage and Gradient stations was typically high, and was only substantially compromised (i.e., by >50 % compared with reference conditions) when total abundances were extreme (i.e. >10,000 per core), at which point low-moderate richness was still maintained (S=15-20, Farm-E, Figure 4-3).

Diversity (H') and AMBI measures were both influenced by the presence of high numbers of opportunists, and consequently provided a more sensitive measure of the enrichment gradient (50-200 m away) than did S or the geochemical measures. Effects to N, H' and AMBI were readily detected out to 200m from the cages, at a point where S, %OM, redox and TFS were comparable to reference site values (Figures 2 & 3, Farms E and F). By contrast, at the low flow sites, all of the variables appeared largely unimpacted beyond 50-100 m from the cages. Similar trends were evident in overall enrichment stage (ES, Figure 4-3), where Farm E and to a lesser extent, Farm F were generally enriched 100-200 m away from the cages relative to the reference sites. Hence, low flow sites were characterised by a benthic footprint that was highly localised but severe in magnitude, whereas high flow sites were less enriched beneath cages, but the overall footprint size was greater.

Natural / un-enriched sediments at the high flow sites were associated with (in reducing order of importance) greater densities of nematodes, Sabellidae, tanaids, Amphipoda, *Spiophanes kroyeri*, *Nucinella maoriana*, Nemertea, *Nucinella maoriana, Goniada* sp., *Maorithyas marama,* Melitidae and *Heteromastus filliformis* (Table 4-3). Several of these taxa were absent from the low flow sites under un-enriched conditions. On the other hand, under natural conditions, the low flow sites were characterised by greater abundances of *Nucula gallinacean, Ennucula strangei* and *Theora lubrica*; in fact, the latter two species were only recorded at low flow sites.

Macrofaunal composition changed markedly with increased enrichment (i.e. increased ES values), with both high and low flow groups reflecting the enrichment associated with

increased proximity to fish cages (Figure 4-4a, b). At comparable high enrichment stages (i.e. ES≈5) the differences between the high and low flow sites were mainly due to proportional abundances of nematodes and *Capitella capitata*, with both taxa being more numerous at the high flow cage sites (Table 4-3). Abundances of *Prionospio* sp. *Phyllochaetopterus socialis, Sphaerosyllis* sp., *Armandia maculata* and *Neanthes cricognatha* were also higher at the high flow than at the low flow sites. At ES 5, nematodes tended to dominate high flow sites, whereas *C. capitata* was the dominant species at the low flow sites. There was no evidence of the high flow sites progressing beyond ES 5 towards an azoic state (ES 6-7), despite feed usage levels being as high (see Table 4-1), and in some cases, higher than at low flow sites where ES 6-7 was clearly evident. In contrast, as the seabed condition beneath cages at low flow sites tended towards azoic the abundance of opportunistic taxa decreased (Figure 4-4a). The relatively low within-group similarity for the low-flow ES 5 samples (40.4%, Table 4-3) primarily reflects the large differences in the relative abundances (i.e. 100's to 1000's) of the two opportunistic taxa that dominated these samples, as opposed to differences in species composition per se.

The single physical variable that best explained the observed macrofaunal groupings at these sites (Figure 4-4b) was 'Current' (ρw = 0.23), followed by '%Mud' (ρw = 0.19) and 'Distance' (ρw = 0.17), with the best combination of variables being current speed and %Mud (ρw = 0.28). Overall, Figure 4-4a,b suggests two main gradients: (i) increasing current and decreasing mud content from bottom left to top right, and (ii) increasing enrichment and reducing distance from cages from bottom right to top left.

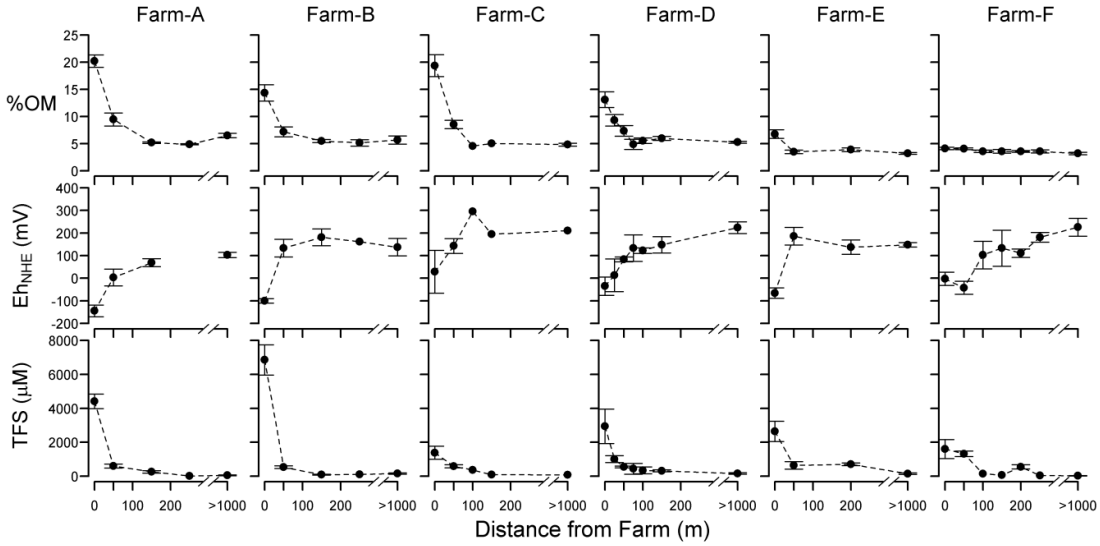

Figure 4-2: Mean values (±SE) obtained for %OM, redox potential and total free sulfides (TFS) from Dataset 2 (for redox and TFS) and Dataset 1 (combined for %OM) averaged across years, plotted in relation to distance from the cages for each farm (A-F).

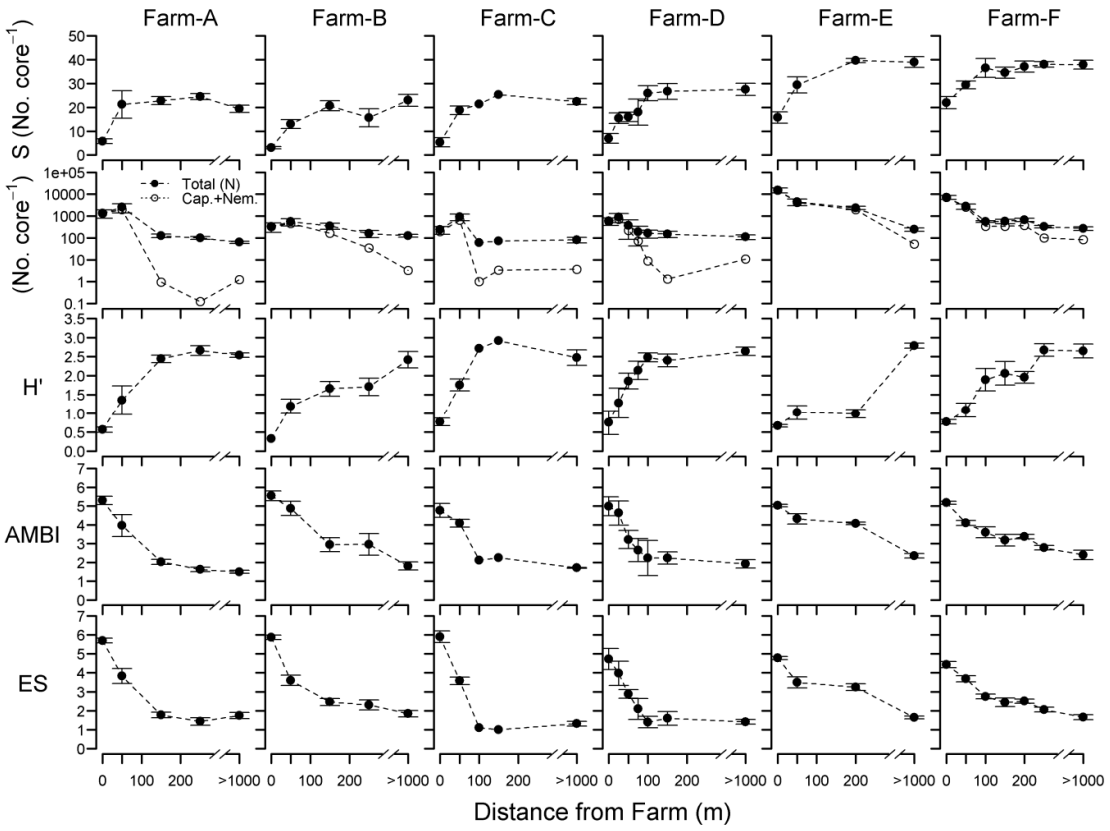

Figure 4-3: Mean values (±SE) obtained for %OM, number of taxa (S), total abundance (N), 'Non-Op' (fraction of non-opportunistic taxa in sample, i.e. excluding *C. capitata* and nematodes), H', AMBI and overall Enrichment Stage (ES) from Datasets 1 and 2 combined (i.e., average across years), plotted in relation to distance from the cages for each farm (A-F).

Table 4-3: Individual taxa that best characterise the groups comprising ES 1 and ES 5, high and low flow data listed along with average abundances, based on SIMPER analysis of 4th-root transformed data.

	Low flow			**High flow**		
ES 1	*Average similarity: 60.94*			*Average similarity: 48.48*		
	Species	**Av.Abund**	**Cum.%**	**Species**	**Av.Abund**	**Cum.%**
	Ostracoda	1.99	10.97	Paraonidae	2.06	6.70
	Cumacea	1.58	19.78	*Heteromastus filiformis*	1.61	11.72
	Ennucula strangei	1.51	28.16	*Prionospio* sp.	1.37	16.71
	Cirratulidae	1.48	36.45	Cirratulidae	1.45	21.68
	Lumbrineridae	1.40	44.25	Cumacea	1.48	26.52
	Paraonidae	1.27	51.07	Nematoda	1.62	31.16
	Nucula gallinacea	1.55	57.75	*Sphaerosyllis* sp.	1.24	35.32
	Ophiuroidea	1.25	64.42	Maldanidae	1.20	39.37
				Tanaid sp.	1.19	42.61
				Nemertea	0.97	45.84
				Ostracoda	1.20	49.05
				Sabellidae	1.19	52.24
				Spiophanes kroyeri	0.99	54.97
				Nucinella maoriana	0.95	57.46
				Goniada sp.	0.89	59.81
				Ophiuroidea	0.90	62.09
ES 5	*Average similarity: 40.42*			*Average similarity: 54.18*		
	Species	**Av.Abund**	**Cum.%**	**Species**	**Av.Abund**	**Cum.%**
	Capitella capitata	4.89	54.52	Nematoda	8.06	30.11
	Nematoda	2.82	71.09	*Capitella capitata*	6.82	55.05
				Prionospio sp.	1.51	60.25

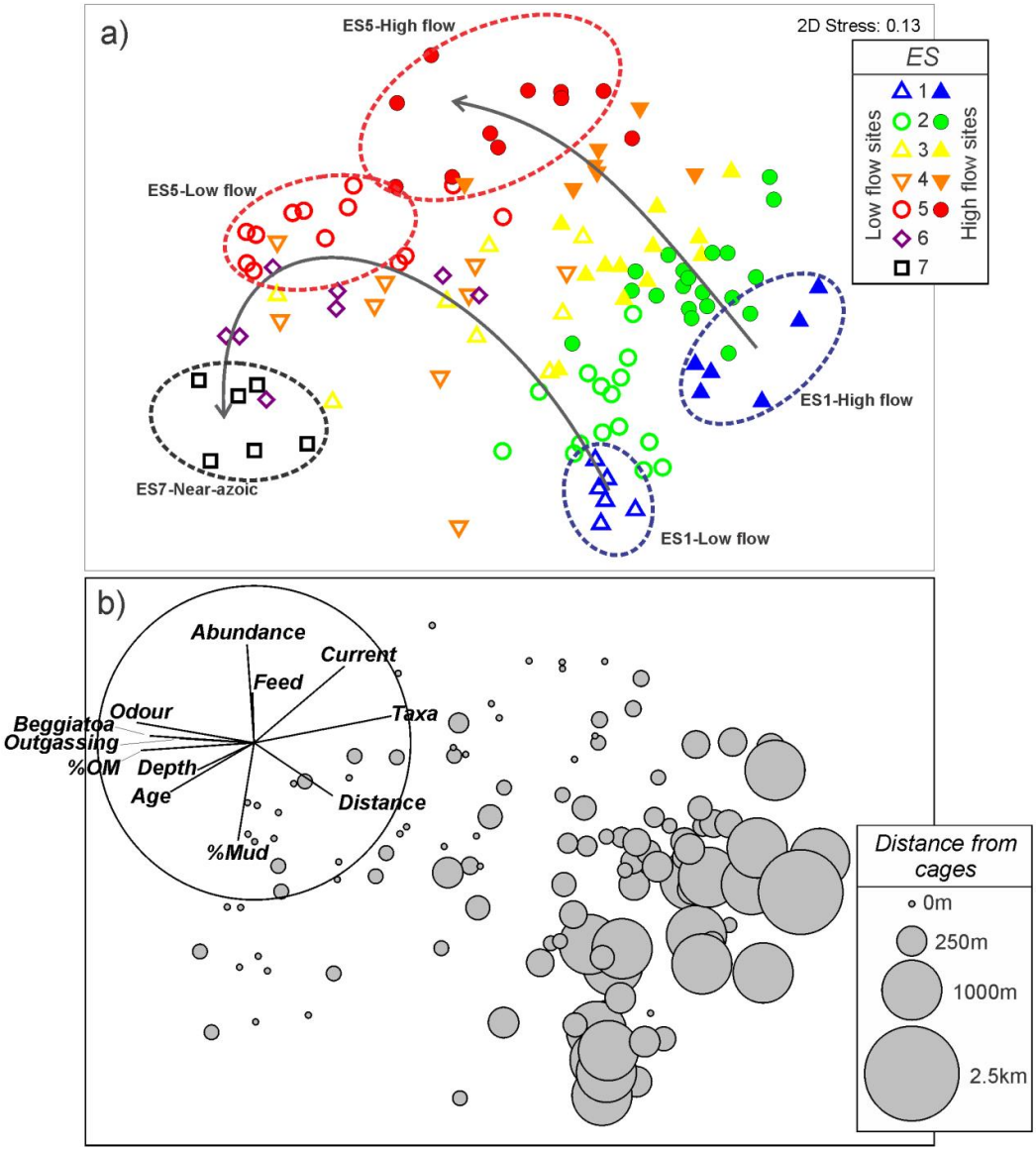

Figure 4-4: Multidimensional scaling (MDS) ordination of benthic macrofaunal assemblages, contrasting those from 3 low flow (Farm A-C) and 2 high flow farms (Farms D & E), based on Bray-Curtis similarities of 4th root transformed abundances: a) MDS ordination with symbols codes according to ES, b) Bubble overlay on same MDS indicating distance of sample from cages, overlaid with Pearson correlation vector showing relationship to associated environmental variables.

4.4.2 Relationship between environmental variables and TFS

Figure 4-5 summarises the relationships between three macrofauna indicators (S, N and H'), ES and redox, with TFS from Dataset 2, and is overlaid with the corresponding relationships identified in Hargrave et al. (2008, 2010). The geochemical relationship between redox and TFS compared favourably between the studies (Figure 4-5e), and was also consistent for low and high flow sites within our study. There was generally a good fit between ES and TFS (Figure 4-5d), with the best models being 2nd and 3rd order polynomials. Values of S and H' at low flow sites were generally not as high in this study as might have been predicted from established models (Figure 4-5a & c), and decreased with increasing TFS, in a manner similar to that described by Hargrave et al. (2010). However, S at the high flow sites remained largely unaffected by TFS until levels approached 2000 µM, at which point there was a clear reduction in number of taxa. As a result, it was not possible to fit a meaningful/ reliable model to the data. Consequently, only categorical criteria can be applied to the relationship between TFS and S, with a 50 % reduction in taxa being associated with levels of approximately 1000 µM TFS, at low flow sites, and 2000 µM at high flow sites. This is broadly consistent with the threshold for reduction in S of 1025 µM proposed by Hargrave (2010).

Total abundance (N) increased in an exponential manner with increasing TFS concentrations (Figure 4-5b). This was particularly evident at the high flow sites, where abundance increased dramatically at TFS concentrations between 500-2000 µM, peaking at >20,000 individuals per core at concentrations >6,000 µM, with no signs of a decline. The relationship provided by Hargrave et al. (2008 and references therein), utilised N values exclusive of first-order opportunistic taxa, and these were similarly removed from our dataset for comparative purposes (denoted N*, Figure 4-5c). In contrast to Hargrave et al., the N* response at the low and high flow sites showed no signs of reduction up until TFS concentrations of 1000 µM; conversely, at the high flow sites N* actually increased slight over this TFS range. At TFS values >1500 µM a sharp decline in N* was evident at the low flow sites, but the same decline was not apparent in the high flow data.

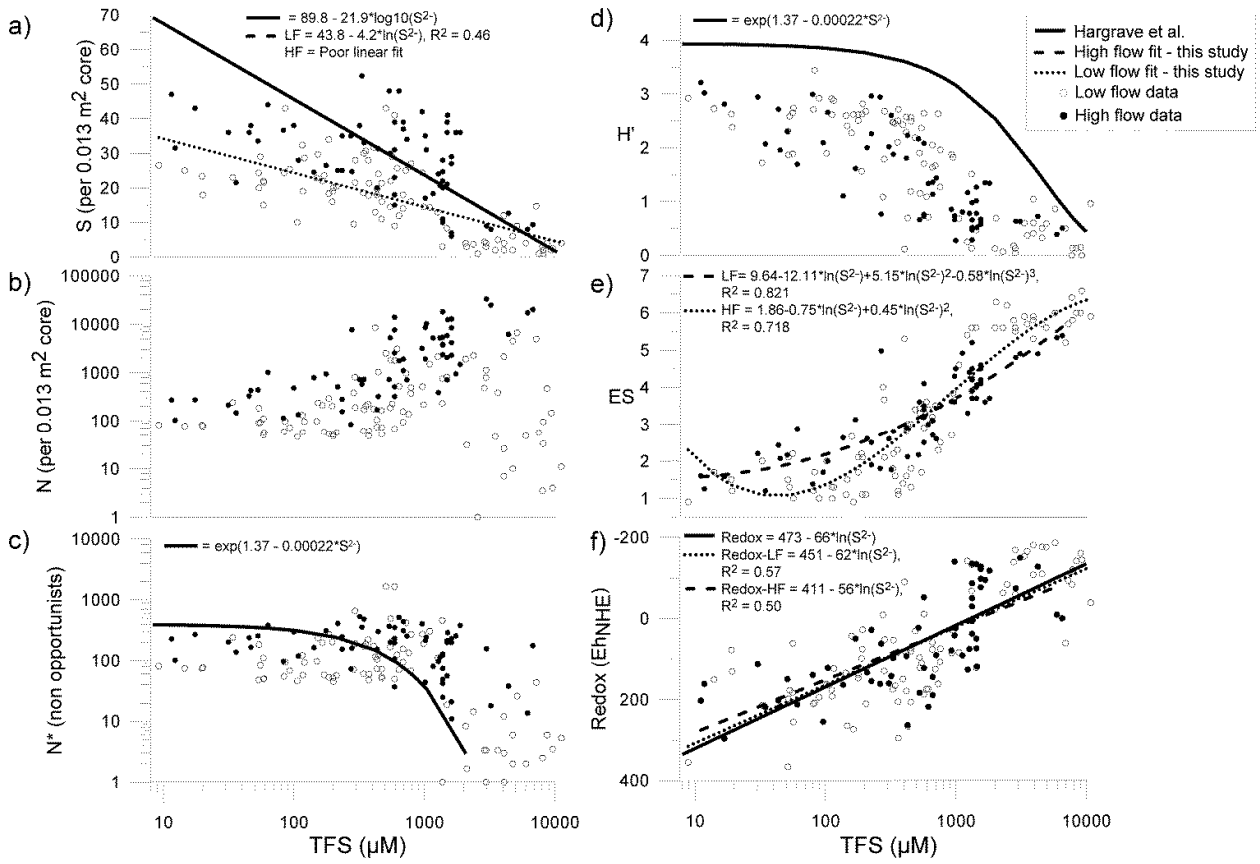

Figure 4-5: Results for: a) No. taxa (S), b) total abundance (N, note the logarithmic scale), c) Abundance of non-opportunistic taxa (N*), d) Shannon Diversity (H'), e) enrichment stage (ES) and f) redox potential plotted against TFS on a logarithmic scale. Utilises Dataset 2, open circles denote low-flow data, closed circles denote high flow data. Black solid lines represent the empirical relationships given by Hargrave et al. (2008, 2010) and Brooks & Mahnken (2003), dashed and dotted lines indicate best-fit least squared regression for high and low flow data, respectively.

4.4.3 Effect of flow regime on abundance, species richness and biomass (SNB): comparison with Enrichment Stage (ES) and the Pearson-Rosenberg Model (PRM)

At the low flow sites, abundance (N) increased gradually with increasing enrichment stage, through ES1-4 before peaking at 7,488 individuals per core (ES 4.6, based on the 95th percentile regression splines; Figure 4-6). A similar pattern was evident at the high flow sites, although abundances were generally much greater, with densities peaking at 81,108 individuals per core. However, the decline observed in N (post-peak, towards ES 6 and 7) at the low flow sites was not evident under high flow conditions, with the pattern following that

shown by the macrofaunal composition changes previously described (see Figure 4-4a). The response of S was comparable at low and high flow sites, with a small initial increase at around ES 2 followed by a progressive decline towards ES 7 (azoic). However, as previously identified, S was generally greater at the high flow sites (peak = 53 taxa as opposed to 37 taxa for low flow sites).

At the low flow sites there were two biomass (B) peaks evident along the enrichment gradient (Figure 4-6); the initial peak at around ES 2 reflected a naturally diverse community, containing a few larger bodied species (e.g. bivalves, ophiuroids, nereid polychaetes). The second, slightly larger peak of 1.64 g $core^{-1}$ corresponded to the peak observed in N (ES 4.7) and was predominantly comprised of opportunistic species (i.e. capitellids and nematodes). Between these peaks B was suppressed, reflecting the absence of the larger bodied individuals. In contrast, B at the high flow sites increased progressively (to >5 g $core^{-1}$) as the overall enrichment level increased, up until ES 5, and was tightly coupled with the near-exponential increase in N (Figure 4-6, Table 4-3). At peak densities, the total macrofaunal biomass at the low and high flow sites was 124 and 378 g m^{-2}, respectively, most of which comprised nematodes and capitellids. The average weights that were determined for individual nematodes and capitellids were 0.05 mg (SE=0.01) and 0.87 mg (SE=0.28), respectively.

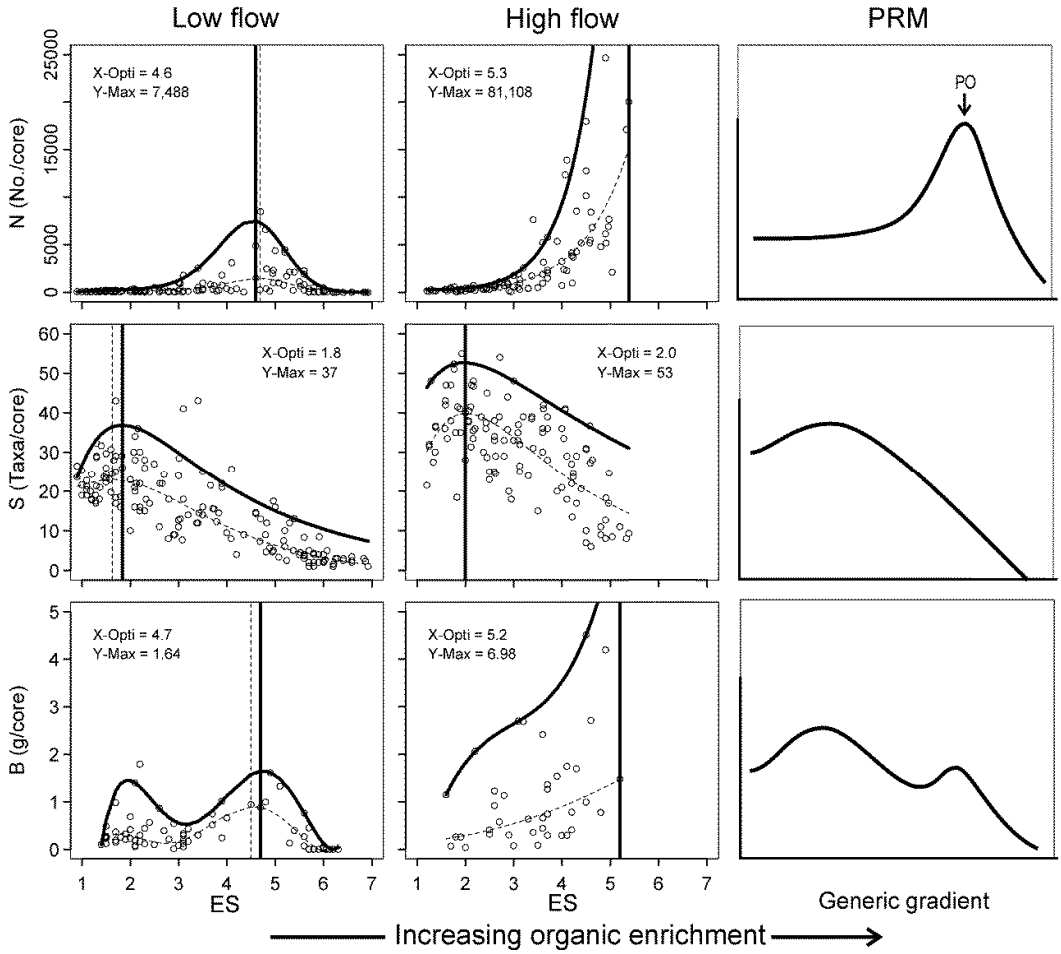

Figure 4-6: Relationships of total abundance (N), number of taxa (S) and total biomass (B), with ES for low flow (left column) and high flow sites (central column) and for the traditional PRM model (right column). Number of taxa and abundance results from Datasets 1 & 2 combined, and biomass results from Dataset 3. Regression splines for the 50th (thin dashed lines) and 95th (thicker solid lines) percentiles with associated estimated optimum (numerically derive peak) indicate by vertical lines.

4.5 Discussion

This study clearly shows substantial differences in successional response along spatial enrichment gradients due to current speed and the associated physico-chemical conditions. Macrofaunal abundances (N) beneath salmon farm cages at high flow sites were nearly an order of magnitude higher than at low flow sites, and to our knowledge are the highest reported in published literature. In a recent study of (10) northern European marine farming sites Borja et al. (2009b) described maximum faunal densities of only 70,000 m^{-2}, whilst a

meta-analysis of benthic enrichment effects comprising 41 independent studies by Kalantzi and Karakassis (2006) reported abundances of up to 500,000 m^{-2}. The maximum densities of 20,000 individuals' per core observed in this study (Figure 3) equate to approximately 1.5 million individuals per m^2. Nematodes comprised a significant component of the taxa recorded, but even with nematodes removed, densities still occasionally exceeded 1 million individuals per m^2, principally due to *C. capitata*. Different populations of capitellids can vary in body size and tolerance of TFS (Gamenick et al. 1998), with the populations encountered in this study appearing to be of the larger TFS tolerant variety. Based on average dry weights of ~0.9 mg per individual (as measured), capitellid worms can represent a significant component of the benthic biomass. Nematodes were less important component as their average individual biomasses were an order of magnitude less than for capitellids.

Extreme abundances of one, or a few, opportunistic taxa is a useful feature for elucidating enrichment effects, as the strong peak provides an important reference point along the enrichment gradient (Pearson & Rosenberg 1978, Glémarec & Hily 1981, Keeley et al. 2012a) and is used as a characterising feature by most diversity measures (e.g. H') and biotic indices (e.g. AMBI and BQI, Benthic Quality Index; Rosenberg et al. 2004). However, at high flow sites, very high abundances often occurred in conjunction with moderate-to-high species richness. A similar macrofaunal response has been described for a high energy open-ocean environment, where an elevated supply of organic material was considered to have a stimulatory effect, as opposed to the adverse eutrophic effect that is commonly associated with poorly flushed systems (Maurer et al. 1993). Under these conditions, the opportunistic taxa have the potential to 'overwhelm' the less-dominant taxa in biotic index calculations, and therefore, the environmental indicators need to be interpreted with caution, particularly with respect to consideration of S.

The observation that %OM was naturally low at the high flow sites, and remained low in the presence of relatively high levels of farm production was not unexpected, as high current velocities are associated with lower carbon sedimentation rates (Findlay & Watling 1997, Cromey et al. 2002b, Giles et al. 2009). Variations in the extent to which %OM accumulates have also been associated with other factors related to near-bottom hydrodynamic conditions, such as sediment composition (Kalantzi & Karakassis 2006, Papageorgiou et al. 2010) and site exposure (Aguado-Gimenez et al. 2007, Macleod et al. 2007, Nickell et al. 2009). Consistent with Nickell et al. (2009), our study clearly shows that community composition can be

markedly influenced (in this case to levels not previously considered), even when there is little or no direct evidence of organic matter accumulation (i.e. evident as %OM). Such findings reinforce previous suggestions that macrofaunal responses are a more sensitive indicator of the overall impact of enrichment than %OM or sediment chemistry variables (e.g., Carroll et al. 2003, Maldonado et al. 2005), and are especially important for reliable monitoring and assessment in well-flushed environments.

The Findlay and Watling (1997) oxygen supply model partly explains these biological conditions, demonstrating how impact, as a result of organic enrichment, can be intrinsically linked to carbon flux and oxygen delivery rates. The model shows how a strong oxygen supply prevents anoxia and associated build-up of H_2S in surface sediments, and in doing so permits greater benthic degradation of the waste stream (Findlay & Watling 1997). In the high flow sites in this study, the benthic community responded to the relatively unlimited supply of organic particulates. However, as a result of the strong hydrodynamics there is effectively no net accumulation, and the physical process of smothering and the subsequent chemical effects on the sediment matrix, which are commonly associated with low-flow muddy sites (Lumb 1989), are kept to a minimum. The same hydrodynamic processes maintain relatively coarse sediments, which have greater oxygen penetration and can support higher benthic diversity than finer silty sediments (Apostolaki et al. 2007, Papageorgiou et al. 2010). We propose that the seemingly anomalous densities of opportunistic taxa observed at high flow sites in the present study are a product of three key environmental factors: i) minimal accumulation of and smothering by fine sediments, enabling naturally coarser sediments to be maintained, ii) maintenance of aerobic conditions in near-surface sediments as a result of water movement and the coarse sediment particle size, and iii) an abundant food supply enabling the proliferation of opportunistic taxa. The combined effect of these factors explains why some commonly applied measures of organic enrichment such as total free sulfides (TFS), redox, S, and *d* (Margalef's richness) are less sensitive indicators in high flow environments (Keeley et al. 2012a).

More detailed analysis of the relationships between biological and geochemical indicators of enrichment and comparisons with other studies further highlights the mismatch in the responses at high flow sites. The relatively strong relationship between TFS and ES generally supports the inference that TFS is a good proxy for overall enrichment stage (Hargrave, 2010); however, the 18-29 % of unexplained variation in the models for our data (R^2=0.82 and 0.71, Figure 4-5d) probably reflects the influence of the other variables that are

used in determining ES (Keeley et al., 2012a). Hargrave et al. (2008, 2010 and references therein) describe the enrichment gradient according to the oxic state and TFS concentrations in the sediments. The main features described were for a normal range of diversity at <300 µM and a transition from oxic to hypoxic conditions ('sub-hypoxic conditions') between 1300 to 1500 µM TFS, at which point *Beggiatoa* mats and opportunistic species appear. Concentrations of 2500 and 3000 µM TFS were regarded as the point at which TFS intolerant species disappeared and tolerant species began to dominate, and then continued to increase up until 5000 µM, beyond which all species decreased in abundance and were depauperate beyond 6000 µM (Brooks & Mahnken 2003a, Hargrave et al. 2008). Although many of these features were evident in the present study in a general sense, there were some notable deviations. At the high flow sites, opportunistic species became dominant at much lower TFS concentrations (700-1000 µM) and continued to increase, seemingly unabated, peaking at 6000 to 7000 µM. The abundance and richness of non-opportunistic taxa at high flow sites were largely unaffected until approximately 1500 µM TFS, at which point a reasonably abrupt decline occurred in both respects. The relationships with TFS at low flow sites were broadly comparable to those described by (Brooks & Mahnken 2003a), however, rather than increasing between 3000 and 5000 µM TFS, abundances of non-opportunists were generally lower and highly variable (0-100 individuals/core).

Some of the discrepancies observed in our study could be attributed to a specific methodological difference. The comparable TFS values provided in Hargrave et al. (2008, 2010) were derived following the methods of Wildish et al. (2004), in which the top 2 cm of the sediment profile is targeted by driving the 5-cc syringe into the sediments on a 45 degree angle. In our study, TFS was determined from the surface 4.5 cm of the sediment, thereby incorporating deeper sediments, which are presumably more highly reduced and have greater TFS levels than the shallower sediments sampled by Hargrave et al. As such, our study may overestimate TFS levels by comparison. The fact that N declines abruptly at relatively high TFS levels at our low flow sites compared to that proposed by Hargrave et al. would tend to support this assertion (Figure 4-5b). That said, irrespective of the potential methodological differences, it is still important to note that total N was positively correlated with increasing TFS at high flow sites up to ~2000 µM, and that abundances of non-opportunistic taxa either remained unchanged (at low flow sites) or increased only slightly (at high flow sites) until TFS reached ~1500 µM. Collectively, these findings highlight that TFS is not necessarily a reliable means to infer biological condition, and as such, concur with earlier findings of Henderson and

Ross (1995). The findings also emphasize the importance of understanding fine-scale depth variations in both biological activity and physico-chemical conditions.

Comparing the observed trends in abundance (N), species richness (S) and biomass (B) at high and low flow sites to the accepted ecological responses to enrichment described by the PRM further highlights flow-specific differences. Aspects of the organic enrichment response gradient defined in the PRM are evident in both the high and low flow data sets, but there are significant deviations under each scenario. Abundance and richness data for low flow sites were, on the whole, consistent with the expectations associated with the enrichment gradient of the PRM. However, the dominant peak in the B curve corresponded to the peak in N, as opposed to the peak in S, as described by Pearson and Rosenberg (1978). Some relatively high B values were recorded in the early stages of enrichment (i.e. ES 2-2.5) due to the presence of a few larger bodied organisms as the PRM would propose and which is typical of a more diverse, healthy fauna.

At the high flow sites, deviations from the PRM were more pronounced, with abundances increasing exponentially with ES but few signs of a subsequent decline, despite comparatively high farm production levels and evidence of degraded biogeochemical properties in the sediments (i.e. TFS and redox). It may be that the sites are yet to be exposed to sufficient flux of carbon; however, it is also conceivable that the physical and biological conditions described above result in increased resilience within the natural communities to the macrofauna 'collapse' which would normally typify ES 6-7. At the high flow sites, B increased dramatically in response to exponential increases in N, and the associated changes in community composition. Although biomass patterns can be influenced by chance sampling of sparsely-distributed large bodied species (Warwick & Clarke 1994), we believe the patterns in our data are reliable, as the samples reflect the integrated results from replicated samples across three surveys and two (high flow) or four (low flow) sites. Consequently, it appears that the assimilation capacity of the benthos at high flow sites may be considerably greater than at low flow sites, and that the maximum capacity may be related to macrofaunal community abundance. This finding is again in contrast to Macleod et al. (2006, 2007) and Mayor and Solan (2011) who found exposed or high flow sites to have a lower assimilation capacity due to deficiencies in the functional traits of the resident organisms. However, in our study the communities at the high flow sites often naturally contained low to moderate abundances of naturally occurring opportunistic taxa, such that the functional benefits of these species (i.e.

their ability to breakdown organic material) may be a key factor underpinning the observed resilience.

4.6 Conclusions and implications

Significantly different organic enrichment effects occur in environments with differing water flow regimes, and in high flow locations, lead to macrofaunal responses that deviate from established conceptual models such as the PRM. Macrofaunal assemblages in high flow environments appear relatively resilient to enrichment, being able to support extreme abundances of opportunistic taxa while simultaneously maintaining moderate species richness. The dispersive nature of high flow environments is clearly an important determinant of ecological responses to enrichment, and will strongly influence the ecological quality status that can be inferred from different environmental indicators.

Percentage OM is a particularly poor indicator of enrichment at high flow sites, and geochemical variables such as redox and TFS can also be poor predictors of biological condition, and therefore such variables should be routinely validated against a full suite of environmental variables inclusive of macrofaunal analysis. Despite flow-related differences in some biological responses at high TFS, abrupt changes in taxa richness and abundance were evident at >1500 µM TFS, which corresponds well to an established threshold for the transition between oxic and hypoxic conditions (Hargrave et al. 2008)et al. 2008). Further work needs to be conducted into how TFS and macrofauna are distributed throughout the surface sediments in high flow environments, as this will further inform decisions regarding the most appropriate sampling methods. Although relatively expensive and time consuming, this study has shown that evaluation of macrofaunal composition and the application of related diversity measures clearly remain the most reliable means of identifying enrichment effects. This appears to be especially true at high flow sites, and when attempting to ascertain the spatial extent of impacts where discriminating between natural and impacted conditions is particularly important.

A key characteristic of the flow regime is that it influences both the per unit area severity of the enrichment effect, and the spatial scale across which effects are discernible. Fish farm biodeposits, or any organic-rich waste for that matter, may have a severe impact at a low flow

site (e.g. lead to azoic conditions), but the impact is typically contained to within tens of metres of the source. By contrast, at a high flow site, the same organic input will lead to a benthic effect that is locally less severe, but may be measureable across scales of hundreds of metres. Moreover, at highly dispersive sites there is increased potential for far-field effects (e.g. deposition in quiescent areas outside of the immediate benthic footprint), and this is an issue that needs more attention. Additionally, high flow sites have been associated with relatively unique communities that can take longer to recover with cessation of farming compared with more conventional low flow sites (e.g., Hall-Spencer & Bamber 2007). Therefore, from an environmental management perspective, the relative merits of the different management strategies must invariably be assessed on a case by case basis. From an aquaculture production perspective, the results suggest that high flow locations may be more resilient to impact for a number of reasons, with this study showing that greater waste loads can be assimilated by the benthos at such locations, potentially enabling production to be increased while maintaining acceptable levels of environmental quality.

4.7 Acknowledgements

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CHAPTER 5

PREDICTIVE DEPOSITIONAL MODELLING (DEPOMOD) OF THE INTERACTIVE EFFECT OF CURRENT FLOW AND RESUSPENSION ON ECOLOGICAL IMPACTS BENEATH SALMON FARMS

Preface:

This chapter focusses on our ability to predict enrichment effects by testing, and subsequently, building on the utility of an existing depositional model (DEPOMOD). The motivation to undertake this work arose because: i) confident model application is contingent upon regional validation, which is especially important where the hydrodynamic properties are relatively unique, and ii) the links between depositional flux rates and observed ecological effects are poorly described. So in this chapter I evaluate the strength of the link between model predictions and observed ecological responses by validating the model at the six study sites. I also develop empirical models to convert between predicted flux and observed effects for dispersive (high flow) and non-dispersive (low flow) sites, and in doing so provide a novel insight to scientific understanding of the role of seabed particle re-suspension.

This work has been published in a refereed journal and has been adjusted to a standard format for the thesis, and as such there may be minor differences in the text, figures and tables compared with the published version. The citation for the original publication is:

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5.1 Abstract

Sediment resuspension is an important factor in controlling the impact of any localised point source impacts such as salmon farms; at high flow (dispersive) sites resuspension can significantly reduce potential effects. DEPOMOD is widely used to predict localised seabed impacts and includes an optional flow-related resuspension module. This study examined the observed impacts at five farms with contrasting flow regimes to evaluate the role of modelled resuspension dynamics in determining impacts. When resuspension was included in the model, net particle export (i.e. no significant net downward flux of organic material) was predicted at the most dispersive sites. However, significant seabed effects were observed suggesting that although the model outputs were theoretically plausible they were inconsistent with the observational data. When the model was run without resuspension the results were consistent with the field survey data. This retrospective validation allows a more realistic estimation of the depositional flux required, suggesting approximately twice the flux was needed to induce an effect level at the dispersive sites equivalent to that at the non-dispersive sites. Moderate enrichment was associated with a flux of ~0.4 and ~1 kg m^{-2} yr^{-1}, whilst highly enriched conditions occurred in response to 6 and 13 kg m^{-2} yr^{-1}, for low and dispersive sites respectively. This study shows that the association between current flow, sediment resuspension and ecological impacts is more complex than presently encapsulated within DEPOMOD. Consequently, where depositional models are employed at dispersive sites validation data should be obtained to ensure that the impacts are accurately predicted.

5.2 Introduction

Aquaculture, and in particular, sea-cage fish farming, is a significant primary industry that is undergoing rapid expansion worldwide. The immediate and obvious environmental impacts associated with finfish farming are well documented (e.g., Gowen & Bradbury 1987, Brooks et al. 2002, Brooks & Mahnken 2003a, Kalantzi & Karakassis 2006). Seabed effects tend to be localised and are typically routinely monitored with the results used to regulate the intensity of the aquaculture activity (Wilson et al. 2009). Depositional models have been proven to be a useful tool for both predicting and managing seabed effects, as they combine physical and behavioural properties of water and particles with farm configuration and production parameters to predict the distribution and intensity of waste products (Cromey et al. 2002a). In New Zealand, as in many other Southern Hemisphere countries, caged fish-farming is a developing industry and accurately predicting impacts and ensuring that farms are properly situated are critical steps in the planning and permitting process.

The numerical algorithms that describe the physical processes underpinning the advection, dispersion and accretion of particles in most deposition models are valid across a wide range of environments, provided the model boundary conditions are adequately described. DEPOMOD (Cromey et al. 2002a) is probably the most established and widely used depositional model for the purposes of predicting salmon farm effects, largely because it has been proven in a wide range of environments and is considered to be robust and credible (SEPA 2005, ASC 2012). Some of the key input parameters that are required, such as observations of current dynamics, bathymetry and basic farming practice information (e.g. cage layout and feed characteristics and input rates) are relatively easy to obtain, whilst others can be more difficult to quantify (e.g. feed wastage, critical erosion thresholds). In these latter cases, default data can be employed as long as the model is not overly sensitive to these parameters. As a result it is possible to transfer a depositional model that has been developed in one environment to another region, often with only minor alterations. For example, although DEPOMOD was developed for salmon farming in cool temperate systems, it has been applied successfully to cod farming (CODMOD, Cromey et al. 2009), and to both warm-temperate culture of sea bream and bass (i.e. MERAMOD, Cromey et al. 2012) and more recently tropical fish-culture (i.e. TROPOMOD). The validation process for these new applications was relatively straightforward and only required site specific data and the

inclusion of a few new processes (e.g. wild fish feeding) - indicating that the physical components were on the whole comparable and transferable.

Although the primary components of the models are generally transferable, the relationship between depositional flux and ecological response can be strongly influenced by physical environmental properties, and is therefore site-specific. Sediment type (i.e. sand versus mud, (Kalantzi & Karakassis 2006, Papageorgiou et al. 2010) and flow regime (Macleod et al. 2007, Mayor & Solan 2011, Keeley et al. 2013a) will each influence ecological responses. Dispersive sites (i.e. with strong currents) will respond characteristically differently to organic enrichment and are potentially more resilient to benthic effects (Frid & Mercer 1989, Borja et al. 2009b, Keeley et al. 2013a), with the total seabed area measurably affected by farming, hereafter termed the 'footprint', often being noticeably larger and more diffuse (Keeley et al. 2013a). Nevertheless, strong biological responses can and do occur at dispersive sites (Chamberlain & Stucchi 2007), as evidenced by very high macrofaunal abundances and biomass in the immediate vicinity of the cages (Keeley et al. 2012a). These differences can largely be attributed to the stronger currents, which increase initial particle dispersal (Cromey et al. 2002b), and provide an increased oxygen supply buffering against near-bottom anoxia (Findlay & Watling 1997). Presumably, greater resuspension also plays an important role, re-entraining and re-distributing particles post-settlement and thereby limiting excessive organic accumulation and related ecological effects (Keeley et al. 2013a). However, the validity of including resuspension in depositional models remains in question, as its' inclusion can strongly influence the results and the optimum critical velocity threshold (vr) to use is debatable (Chamberlain & Stucchi 2007).

The ability to clearly and quantitatively link predictions of depositional flux to predictions of ecological effects would greatly increase the usefulness of depositional models. Connecting the mathematical theory and the ecology is essential if the models are to be used for managing farms in relation to benthic effects, i.e. setting maximal and optimal feed levels and/ or fine scale positioning of cages. Studies have been conducted with respect to relatively unique and sensitive communities such as Maerl beds (Sanz-Lazaro et al. 2011) and seagrass habitats (Apostolaki et al. 2007, Holmer et al. 2008), or assessing lower tolerance thresholds, where impacts are initially observed (Hargrave 1994, Findlay & Watling 1997, Chamberlain & Stucchi 2007, Cromey et al. 2012). These studies suggest ecological effects can be observed across a broad range of depositional flux levels spanning two orders of magnitude (i.e.

between 0.1 and 10 kg solids m^2 yr^{-1}), and the results are difficult to compare due to differences in the enrichment criteria or ecological thresholds that have been adopted. Additionally, efforts to relate deposition to benthic responses empirically, have focussed on a relatively limited suite of biological indictors, e.g. total macrofaunal abundance, the infaunal trophic index (ITI) (Cromey et al. 2002a), and biomass, Shannon-Weiner diversity (H') and the biological fraction index (BFI) (Cromey et al. 2012). However, relationships with other biotic indices that can be more effective for discerning benthic enrichment status are yet to be established (e.g. AMBI, Multivariate-AMBI and BQI; Borja et al. 2009b, Keeley et al. 2012a).

Hence, the main aim of this study was to utilise a long-term benthic monitoring dataset to develop empirical models that can be used to convert between predicted flux and observed effects for dispersive and non-dispersive sites, and in doing so contribute to our understanding of the role of resuspension. As a component of this study, it was also necessary to evaluate the strength of the link between model predictions and observed responses by examining the fine-scale differences between the overall size, shape and intensity in the predicted and observed depositional footprints.

5.3 Methods

5.3.1 Study sites and environmental data

The study uses data obtained from annual compliance monitoring program over 12 years (1998 – 2010) at six salmon farms located within the Marlborough Sounds, New Zealand (Figure 5-1). The farms were situated in comparable depths (27 – 40 m) and spanned a range of ages (1 and 25 years of operation, Table 5-1). Four of these farms (A – D) had mean current velocities below 9 cm s^{-1} at 20 m water depth (approximately mid-water), and these are hereafter referred to as 'non-dispersive' sites, whereas the other two (E and F) had mean current velocities in excess of 15 cm s^{-1}, and are referred to as 'dispersive' sites. All of the sites are situated over unconsolidated sediments; the non-dispersive sites tending to be sandy-mud (55 – 91 % mud), and the dispersive sites muddy-sand (28 – 32 % mud; Table 5-1). All of the sites had, at some point, displayed strong enrichment gradients with proximity to the farms (Keeley et al. 2012a, Keeley et al. 2013a). The analyses presented here were conducted on a

deliberately broad range of scenarios, whereby the years that were used for each farm were selected to span a wide cross-section of total annual feed inputs and therefore presumably, associated levels of impact (Table 5-1).

Sediment samples were collected from directly beneath cages, and at stations along an enrichment gradient extending away from the cages (25 – 250 m), as well as at references stations. Macrofauna were sampled using replicate (n = 2, 3 or 5, depending on year of survey) Perspex sediment corers (13 cm diameter, 0.013 m^2) deployed to a depth of 10 cm. Core contents were sieved to 0.5 mm, and the retained fauna identified to the lowest practical taxonomic level and enumerated, enabling calculation of a variety of community composition statistics and biotic indices: N (total abundance), S (number of taxa), H' (Shannon-Weiner diversity), AMBI (Borja et al. 2000) and BQI (Rosenberg et al. 2004). The surface 3 cm of smaller sediment cores (7 cm diameter) was collected for analysis of grain size and total organic matter (OM). Sediments were oven-dried to constant weight at 105ºC, and size class fractions from silt-clay through to gravel were analysed gravimetrically. Percentage OM (%OM) was calculated as the % weight loss of dried samples after ashing at 550ºC for 2 h (modified after Luczak et al. 1997). Redox potential (Eh_{NEH}, mV) and total free sulfide (TFS, µM) were also routinely measured post-2008. Redox was measured directly from the grab (at 1 cm depth) using a Thermo Scientific combination Redox/ORP electrode. Total free sulfide (TFS, µM) was sampled with a cut-off 5-cc plastic syringe driven vertically into the surface sediments (0-4.5 cm depth interval), and the TFS contents were extracted and quantified following the methods of (Wildish et al. 1999).

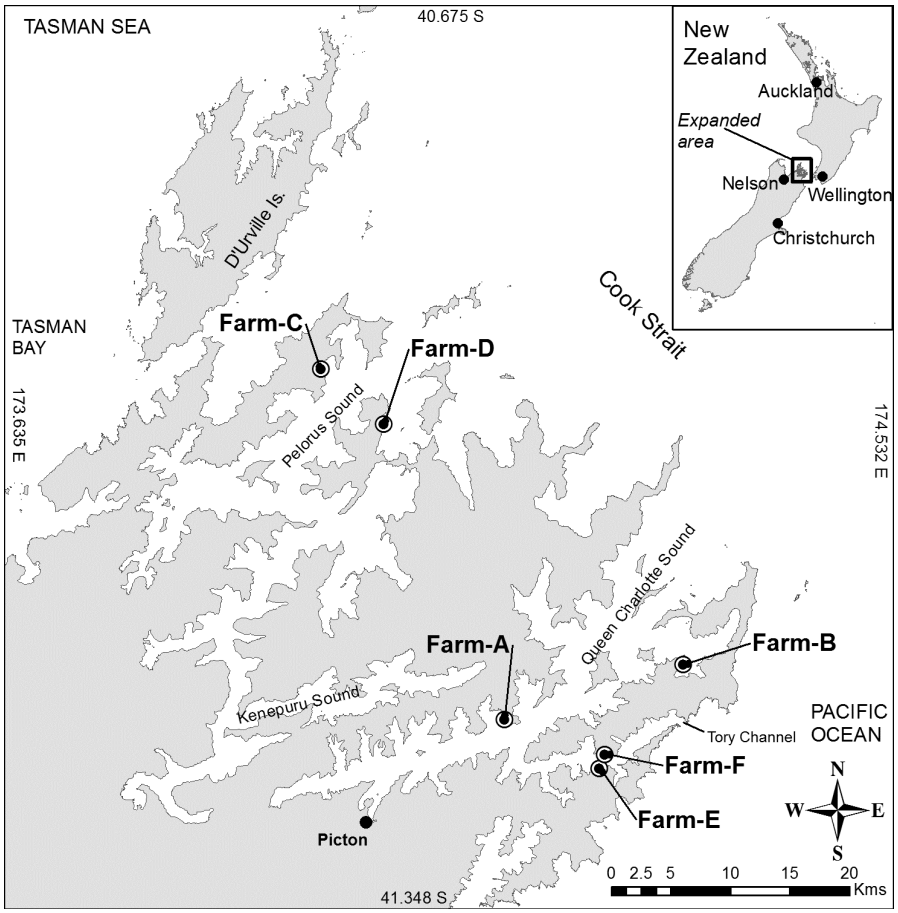


Figure 5-1: Location of study sites in Marlborough Sounds, New Zealand.

Table 5-1: Summary of farm configurations, historical use and physical attributes used in models, and of natural (Reference site) sediment characteristics for each.

Site attributes	Units	Farm-A	Farm-B	Farm-C	Farm-D	Farm-E	Farm-F
Year of survey	20-	'04,'06,'09,10	'05,'08,'10	'03,'05,'09	10	'05,08,'09,'10	'08, '09, '10
Farm age	Year	19,21,24,25	16, 19, 21	14, 16, 20	1[†]	13,16,17,18	1,2,3
Corresponding feed levels	kt·yr^{-1}	1.9,3.3,2.2,2.0	2.2,2.0,1.9	2.1,2.6,3.1	3.3	2.3,4.1,3.9,4.7	2.8, 3.1, 3.5
Site depth range	m	34-35	37-39	28-30m	32-35	27-31	30-40
Mean current speed							
Depth 1 (near-surface)	m: cm s^{-1}*	1: 3.6(30.0)	1: 8.6(35.9)	1: 11.9(59.1)	1: 3.4(16.1)	1: 18.7(62.8)	2: 20.4(87)
Depth 2		7: 4.0(21.6)	9: 3.7(46.1)	7: 8.2(34.3)	8: 3.0(9.3)	7: 16.7(59.2)	10: 20.2(85)
Depth 3 (mid-water)		15: 3.7(17.5)	16: 6.0(34.6)	14: 8.2(29.9)	16: 3.0(10.1)	14: 14.4(53.8)	18: 19.9(117)
Depth 4		22: 3.1(12.9)	26: 9.7(30.4)	21: 8.5(30)	24: 3.2(11)	21: 13.9(42.4)	28: 19.7(129)
Depth 5 (near-bottom)		30: 3.5(14.2)	34: 3.6(13.5)	28: 9.1(29.1)	32: 3.2(10.9)	26: 15.9(49.8)	36: 19.5(79)
ADCP sample bin size	m	4	4	1	1	1	2
ADCP sampling interval	min	45	45	30	30	30	15
Deployment season	Month	January	March	July	February	August	October
Flow category		Low	Low	Low-Mod	Low	High	High
Sampling stations (Distance from cages)	m	0(×2)[‡],50, 150,250,Ref	0(×2),50, 150,250,Ref	0(×2),50(×2), 100,Ref	0(×2),25,50, 75,100,150, 200,Ref	0(×2), 50, 100, Ref	0(×2),50(×2), 100(×2),150(×2), 200(×2),250(×2),Ref
Natural sediment properties[+]							
Sediment mud content	%	80 (69-84)	55 (34-73)	78 (69-85)	91(84-95)	28 (21-38)	32 (29-37)
%OM	% w/w	5.2 (4.8-5.8)	5.0 (2.8-7)	4.9 (4.5-5.8)	5.5(4.4-6.5)	3.1 (2.5-3.7)	3.3 (2.5-4.2)
No. taxa	No./core	22 (18-28)	18 (17-19)	20 (16-23)	21(11-26)	35 (27-48)	39 (31-42)
Macrofauna abundance	No./core	107 (76-147)	72 (52-92)	78 (37-128)	54(18-72)	218 (152-285)	231 (102-278)

[†] Farm had been reinstated for one year at time of monitoring after 8 years of recovering since being fallowed in 2001.

[*] Depths are relative to surface, current speeds are means with maximum speed in brackets.

[+] Mean values and ranges (min-max) from the reference sites that were sampled during the selected surveys for each farm.

[‡] '×2' denotes two separate sampling stations for the give position.

5.3.2 Bathymetry and hydrography

Bathymetry was established for each site and the xyz data was gridded to the desired size and resolution using Surfer v9 for incorporation into DEPOMOD. Model grid sizes were set such that they would comfortably encompass the whole initial depositional footprint (grid areas ranged from 0.23 km^2 for Farm-C to 1.1 km^2 for Farm-A). Water currents were measured using Acoustic Doppler Current Profilers (ADCP, Sontek, 500 kHz) every 15, 30 or 45 minute intervals over 25-42 days. ADCPs were bottom-mounted within approximately 30 m from the cage edge and sampled the water column in 2 or 3 m depth bins (with a 1 m blanking distance). Current data was converted to hourly-averaged bins, and the five depth bins that evenly spanned the full water column at each site (i.e. from near-surface, to near bottom) were selected for use in the models (Table 5-1).

5.3.3 Model parameters

DEPOMOD was selected because it is widely used and published, and designed specifically for managing fish farm wastes (Cromey et al. 1998, Thetmeyer et al. 2003, Cromey & Black 2005, Cook et al. 2006, Magill et al. 2006) and a number of the processes in DEPOMOD have already been validated against field measurements (Cromey et al. 2002a, Chamberlain & Stucchi 2007). It is also used as a regulatory tool in Scotland for discharge discharge of in-feed chemotherapeutants, and in setting biomass limits (SEPA 2005) and is the model that is recommended for predicting seabed effects by the Aquaculture Stewardship Council (ASC 2012).

Standard feed wastage (F_{wasted}) of 3 % was used for all sites and all years in the absence of any reliable historical estimations. This level was selected because it represents a compromise between the level of 5 % shown to support predictions in other studies (e.g., Cromey et al. 2009, Cromey et al. 2012), and the level most recently determined in local studies (<1%, Cairney & Morrisey 2011). Three percent is also the level currently recommended by the Scottish Environmental Protection Agency for regulatory modelling of fish farms in Scotland (Annex H, SEPA 2005). Feed digestibility (F_{dig}) and water content (F_w) were set at 85 % and 9 %, respectively, which are the DEPOMOD defaults based on technical data provided by feed manufacturers (Cromey et al. 2012) and were used in the absence of farm and time-specific estimates. All other model parameters were consistent with existing

salmon farm waste modelling methodologies (Cromey et al. 2002a, Cromey et al. 2002b) and the SEPA Annex H regulatory farm modelling standards (SEPA 2005) and remained constant in the tested model scenarios (Table 5-2). As the model does not allow the settling velocity of particles to change through the growing cycle, the values used for feed and faeces represented those that would be encountered during the period of highest waste output from the farm (maximum standing biomass), which is when the fish are at pre-harvest size.

Table 5-2: Default model settings that were applied consistently throughout the modelling. *Kx, ky* and *kz* are horizontal and vertical dispersion coefficients.

Input variable:	Setting
Feed wastage:	3 %
Water content of feed pellet:	9 %
Digestibility:	85 %
Settling velocity of feed pellet:	0.095 m s^{-1}
Settling velocity of faecal pellet:	0.032 m s^{-1}
Random walk model:	
kx, ky:	0.10 m^2 s^{-1}
kz:	0.001 m^2 s^{-1}

Feed input data was based on total feed used per farm per month and was spread evenly across all cages. In practice, one or two cages may be empty for short periods of time as a result of operational requirements, however this resolution of spatial and temporal information was not available and would in any case be impractical to include in the model. However, this represents a potential source of variability in the outputs, which was accounted for by taking the average result from multiple scenarios. The farm management conditions for each scenario (i.e. number of cages, net depths, overall size and position of farm and monitoring stations) were determined from information collected during annual monitoring surveys (e.g. GPS fixes of farm corners), historical aerial and satellite images, and discussions with farm operators. The standard farm configurations involved square cages with a net depth of 20 m arranged in adjoining clusters, either one or two cages wide and four to eight cages long.

Depositional flux was predicted for 110 benthic sampling locations, representing 18 different historical farming arrangements, encompassing all six study farms (Farms A-F) over eight years (2003 to 2010, Table 5-1). Results were obtained for four different feed levels

based on the average reported feed use for the 1, 3, 6 and 12 months immediately prior to the environmental data being collected. Four critical resuspension velocities were contrasted within each average feed use period: i) without resuspension, and with resuspension based on critical velocity thresholds (vr) of: ii) 9.5 $cm \cdot s^{-1}$ (model default), iii) 12 $cm \cdot s^{-1}$ and iv) 15 $cm \cdot s^{-1}$. Thus, 16 model runs were conducted for each of the 18 different farming scenarios, giving a total of 288 runs. Matlab™ code was developed to enable batch processing of model runs.

5.3.4 Relating predicted flux to observed Enrichment Stage

Environmental condition was determined using established ecological indicators: N, S, H', AMBI and BQI in combination with physico-chemical variables (%OM, redox, TFS). All variables were also unified following the methods of Keeley et al. (2012a, 2012b) to obtain an indication of overall Enrichment Stage (ES); a bounded continuous variable that places the results on a scale between ES1 = 'pristine' to ES7 = azoic/ anoxic. Generalised additive modelling was then used to establish the relationship between predicted flux and observed ecological responses, as shown by ES and each the individual indicator variables.

Prior to analysis, both predicted flux and ES values were log transformed to improve data normality and reduce heteroscedasticity. The necessity to construct flow-specific models was checked by testing the significance of 'flow' as a fixed factor (High / Low) using linear models in R (R Development Core Team 2011). In all cases factor 'flow' was highly significant ($p < 0.0001$). The optimum linear model for each was then identified by fitting four different polynomials (of order 1 to 4) and then selecting the model with the smallest AIC (Akaike's Information Criterion) value. If the AIC values of two models were within 2 units (and could therefore be considered equivalent, Burnham & Anderson 2002), then the simplest model was chosen in preference. The best-fit polynomials were solved for x (or ES) to obtain estimates of the average flux associated with ES3 (i.e. ES=3) and ES5 (i.e. ES=5), and the standard errors of the coefficients were used to calculate the associated 95% pointwise confidence bounds (hereafter referred to as confidence intervals, or 'CI'). ES3 was selected to represent the outer boundary of effects because this level is considered indicative of the point at which enrichment becomes clearly discernible, whilst ES5 indicates the point of peak infauna abundance, and characterises a highly enriched state (Keeley et al. 2012a).

5.3.5 Model validation: spatial comparison of predicted and observed footprints

The footprints of the two high-flow, dispersive farms (Farms E & F) and one low flow, non-dispersive farm (Farm A) were mapped from 79, 65 and 96 grab sampling stations (respectively) collected across a grid pattern spanning the sediments within 1.5 km of the cages. In all cases the density of the sampling grid decreased with distance from the farm in a stratified manner to ensure that sampling effort was greatest where changes in the footprint were expected to be most pronounced (Figure 5-2). These farms were selected because i) they had similar farm layouts, and ii) had consistent usage patterns (cage deployment and feed input) in recent years. They also share similar physical attributes (i.e. depths & exposure), but vary significantly in their typical range of current speeds (Table 5-1). Only three farms were able to be surveyed because of logistical and financial constraints.

Enrichment was assessed at all sampling stations using three proxy variables: i) sediment redox (Eh_{NHE}, mV), ii) sulfide (S^{2-}, µM) levels, and iii) odour. Odour was assessed consistently by the same person using five categories: 1=none, 2=mild, 3=moderate, 4=strong, 5=very strong. Approximately twenty stations at each farm, representing the full range of conditions (i.e. from alongside cages to the most distant reference site) were selected for more comprehensive condition assessments, comprising: macrofauna evaluation, sediment grain size characterisation and %OM content following the methods described above (Section 2.1). The three proxy variables were combined multivariately using principle component analysis (PCA, in PRIMER v5, Clarke 2006) based on Euclidean distances. Sulfide and redox data were log-transformed and all variables were normalised prior to analysis. The Eigenvalues of the dominant PCA axis were used to quantitatively differentiate the sampling stations. ES was also determined for each of the comprehensively sampled stations using a combination of the empirical relationships derived by (Keeley et al. 2012a) and best professional judgement.

The linear regression that best described the relationship (based on highest residual R^2 values) between the Eigenvalues (based on redox, sulfides and odour) and the ES score was determined for each farm survey. These regressions were then used to estimate ES for all stations based on the Eigenvalues, and the results interpolated using the Kriging method (Isaaks & Srivastava 1989) before being spatially depicted in Surfer™ (v9). Finally, the measured footprint was compared to the predicted footprint by converting the predicted flux for the corresponding farm scenario to ES using the best-fit relationships that were identified

from modelling the historical farming scenarios. ES3 was selected to indicate the outer boundary of effects for the reasons given in the previous section.

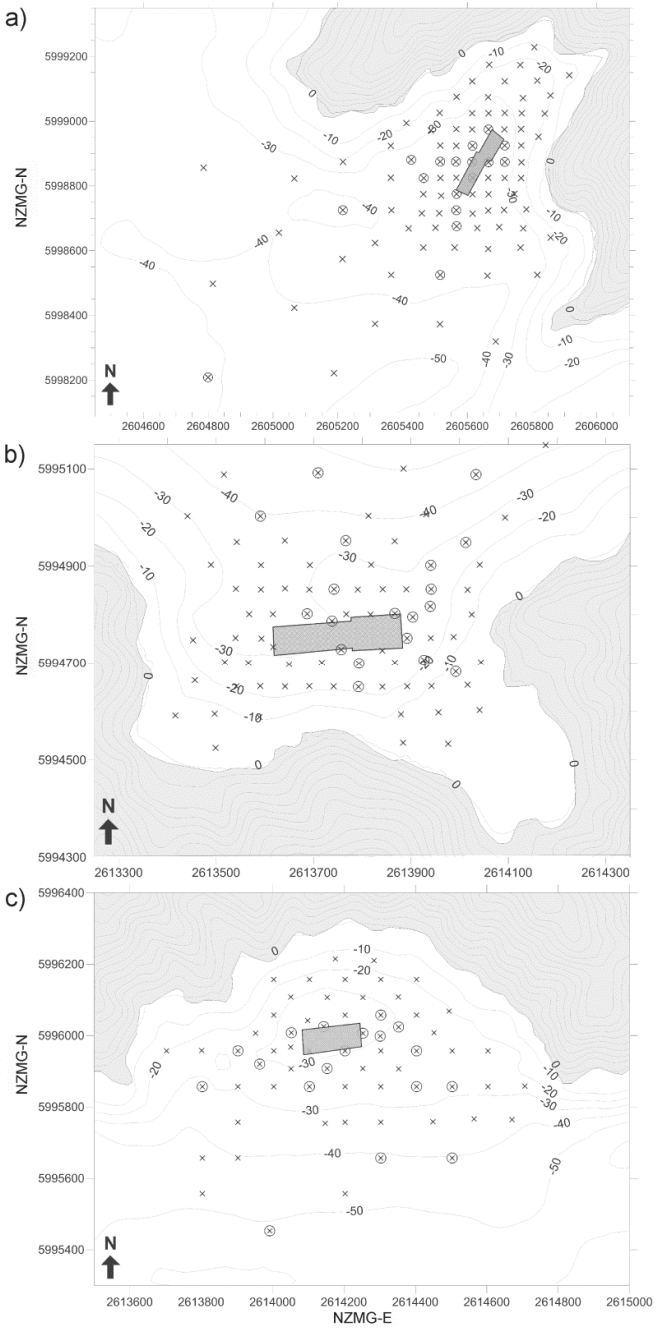


Figure 5-2a-c: Sampling grids that were used to map the enrichment footprints at the two high-flow study sites, Farms A (a), E (b) and F (c). '×' denotes where the three proxy variables (redox, S^{2-}, odour) were sampled and '⊗' indicates those stations at which more comprehensive sampling was conducted (i.e. including macrofauna, sediment grain size and %OM). Grey box denotes position of net pens. Axis units are in meters – East and North along conventional New Zealand Map Grid (NZMG).

5.4 Results

5.4.1 Relating predicted fluxes to observed ecological responses

The central tendency of the relationships between observed ecological responses (as indicated by ES) and the predicted depositional flux (as the explanatory variable), without resuspension, was best described by first and second order polynomials on log transformed data (Figure 5-3, Table 5-3). The best model fit for the non-dispersive sites was obtained with the feed levels applied over the 6 months preceding the respective sampling events (R^2 = 0.898). However, the differences between the three four time series scenarios (i.e. 1, 3, 6 and 12 months prior), were small, with R^2 values of between 0.869 and 0.890 (Figure 5-3A-D, Table 5-3). A moderate / detectable level of enrichment (i.e. ES3) was associated with an average predicted flux of between 0.33 (CI: 0.27, 0.4) and 0.35 (CI: 0.3, 0.44) kg solids m^{-2} yr^{-1}. Very highly enriched conditions, indicative of peak macrofauna abundance (i.e. ES5), were associated with modelled depositional fluxes of between 5.6 (CI: 3.7, 9.2) and 6.3 (CI 4.2, 10.6) kg solids m^{-2} yr^{-1} (Table 5-3).

The model fits for the dispersive sites without resuspension had slightly lower R^2 values than for the non-dispersive sites, with results for the four feed levels ranging between 0.73 and 0.78 (Table 5-3, Figure 5-3A-D). The modelled fluxes associated with ES3-type conditions at the dispersive sites were higher than at the non-dispersive sites, with fluxes ranging between 0.75 (CI: 0.44, 1.64) and 1.15 (CI 0.67, 2.65) kg solids m^{-2} yr^{-1} (Table 5-3). Similarly, the average predicted flux associated with ES5-type conditions was approximately two-fold higher for dispersive sites than for the non-dispersive sites, with estimates of between 12.1 (CI: 5.9, 81.1) and 15.6 (CI: 6.9, 231) kg solids m^{-2} yr^{-1}. However, the upper confidence intervals for these estimates were very high due to increased variation at the upper end of the enrichment gradient and the log-relationship between Flux and ES.

Where resuspension was taken into account (Figure 5-3E-G) the model outputs were comparable with the no-resuspension results for non-dispersive sites. Although the overall fit with the observed data was worse, this improved from R^2 = 0.65 to R^2 = 0.88 as the critical resuspension velocity increased from 9.5 cm s^{-1} (model default) to 15 cm s^{-1}. The poorer fit where resuspension was included in the scenario was primarily due to the predicted fluxes for some of the moderately enriched stations (i.e. ES3-5) at Farm-C (which has the highest current

speed of the two non-dispersive sites) being zero. At the dispersive sites, the net depositional flux was predicted to be zero for all three critical resuspension velocities, even at stations that were directly beneath the cages. As a result, no meaningful relationship could be derived between flux and effects for those scenarios.

The relationships between predicted depositional flux and individual response variables were generally not as strong as the relationships with the multi-variable derived ES (Figure 5-4). However, H' and AMBI were both reasonably well predicted by the models at both dispersive and non-dispersive sites (R^2 = 0.56 - 0.76, Table 5-4). Number of Taxa (S) and BQI were poorly predicted by depositional flux at the dispersive sites (R^2 = 0.27 and 0.31 respectively), but well predicted at the non-dispersive sites (R^2 = 0.78 and 0.56 respectively). Conversely, log(N) was related to predicted flux at the dispersive sites (R^2 = 0.65), but not at the non-dispersive sites (R^2 = 0.07); the former being best described by a more complex fourth-order polynomial.

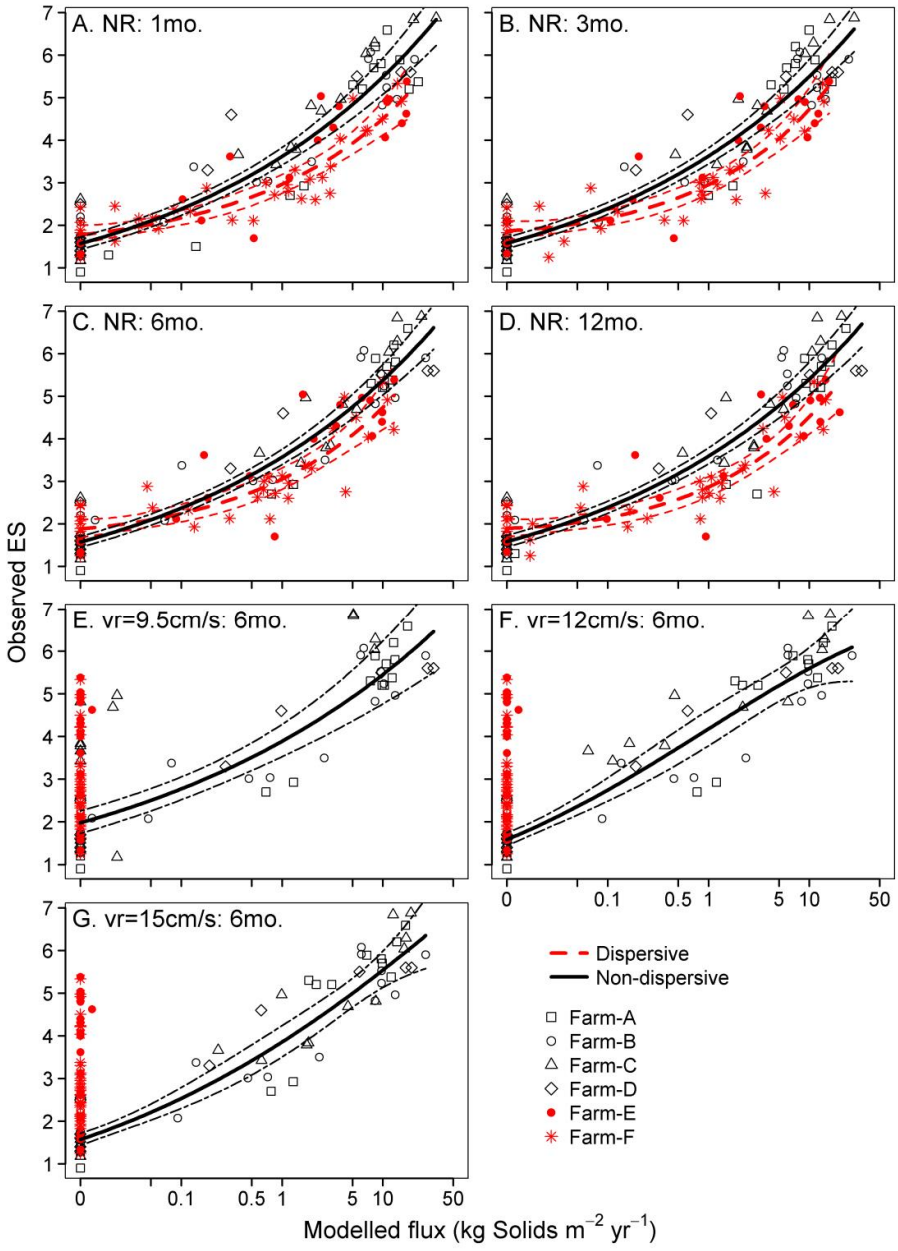


Figure 5-3: Log-log relationships for predicted depositional flux and observed enrichment responses (as the response variable) at sampling stations associated with 4 low-flow (Farms A-D) and 2 high-flow farms (Farms E and F). Equations and model fits parameters are provided in Table 5-3. Thin dashed lines show 95% pointwise confidence bounds for the fitted curves. 'NR' – no resuspension, 'vr' – critical resuspension threshold used, and 'mo.' – temporal scale of integrated feed input data.

Table 5-3: Summary of polynomial coefficients and fits for relationships between predicted depositional flux and observed ES (as the response variable). Average flux (kg solids m^{-v2} yr^{-1}) required to induce ES3 (moderate, detectable enrichment) and ES5 (very high enrichment defined by peak of opportunists) provided along with upper and lower confidence interval (in brackets). 'Feed' = period preceding field sampling over which feed use was averaged, 'vr' = critical velocity for resuspension, cm s^{-1}, NR = no resuspension, 'Deg.' = degree polynomial, RSE: residua standard error, Note: no meaningful relationship could be derived between flux and effects for results from dispersive sites with resuspension taken into account (see Figure 5-3).

Feed	vr	Deg.	Int.	ln(x+0.01)	ln(x+0.01)^2	RSE	df	R^2	P-value	ES3 (CI's)	ES5 (CI's)
Non-dispersive:											
1	NR	1	1.3	0.18		0.2126	56	0.869	<0.001	0.33 (0.28, 0.42)	5.72 (3.68, 9.81)
3	NR	1	1.3	0.18		0.1949	56	0.89	<0.001	0.33 (0.27, 0.4)	5.65 (3.77, 9.2)
6	NR	1	1.28	0.18		0.187	56	0.898	<0.001	0.35 (0.3, 0.44)	6.36 (4.26, 10.26)
12	NR	1	1.29	0.18		0.1968	56	0.888	<0.001	0.34 (0.28, 0.42)	6.15 (4.05, 10.19)
6	9.5	1	1.37	0.15		0.3468	56	0.651	<0.001	0.2 (0.12, 0.23)	5.2 (2.33, 17.25)
6	12	1	1.44	0.15		0.2101	55	0.872	<0.001	0.1 (0.08, 0.15)	3 (1.49, 7.72)
6	15	1	1.36	0.17		0.197	55	0.887	<0.001	0.2 (0.16, 0.31)	4.4 (2.23, 11.18)
Dispersive:											
1	NR	2	1.09	0.152	0.01	0.207	48	0.723	<0.001	0.91 (0.57, 2.17)	15.65 (6.97, 231.9)
3	NR	2	1.08	0.163	0.014	0.2127	48	0.707	<0.001	0.93 (0.59, 2.05)	12.1 (5.98, 81.07)
6	NR	2	1.12	0.154	0.011	0.2196	48	0.688	<0.001	0.75 (0.44, 1.64)	12.2 (5.54, 198.5)
12	NR	2	1.06	0.155	0.014	0.2096	48	0.716	<0.001	1.15 (0.67, 2.65)	14.72 (6.99, 103.5)

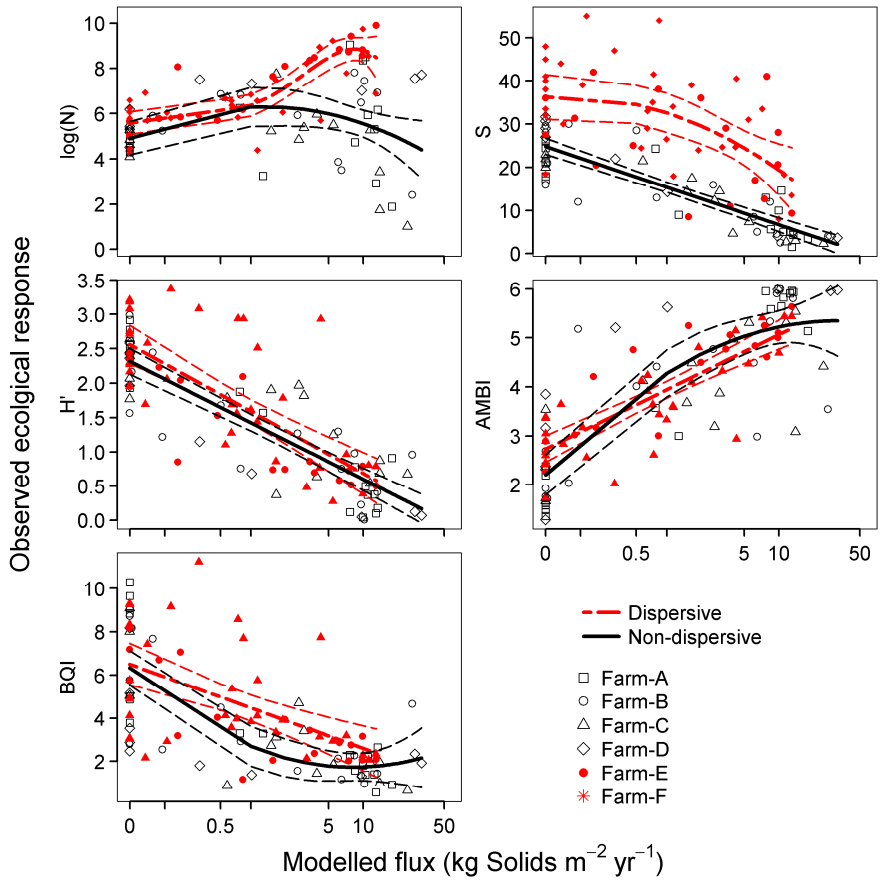


Figure 5-4: Relationships between predicted (log) depositional flux and five enrichment indicating biological variables. Equations and model fits are provided in Table 5-4.

Table 5-4: Summary of polynomial coefficients and model fits (R^2) for relationships between predicted depositional flux and five biological enrichment indicators. Deg. = degree of best-fit polynomial, Int. = intercept, RSE = residual standard error, df = degrees of freedom.

	Variable	Deg.	Int.	x	x^2	x^3	x^4	RSE	df	R^2	P-value
Dispersive	log(N)	4	6.86	8.06	1.91	-0.32	-2.1	0.861	46	0.654	<0.001
	S	2	30.72	-42.51	-15.06			9.824	48	0.276	<0.001
	H'	1	1.69	-4.94				0.613	49	0.561	<0.001
	AMBI	1	3.82	6.14				0.600	49	0.674	<0.001
	BQI	1	4.69	-10.28				2.113	49	0.311	<0.001
Non-dispersive	log(N)	2	5.41	1.1	-3.99			1.642	55	0.071	4.9E-02
	S	1	14.65	-63.96				4.478	56	0.780	<0.001
	H'	1	1.35	-6.05				0.418	56	0.762	<0.001
	AMBI	2	3.97	10.06	-1.93			0.924	55	0.679	<0.001
	BQI	2	3.59	-14.81	4.8			1.789	55	0.564	<0.001

5.4.2 Model validation: spatial comparison of predicted and observed footprints

The primary axis of the PCA analysis (i.e., PC1), integrating the three proxy variables (redox, S^{2-} and odour) was a good indicator of the overall variation between stations at Farms E and F (N = 64 and 84, %Variation described by PC1 = 84 and 85%, respectively). The resulting PC1 values also correlated well with the ES scores determined from the 18-19 samples for which infauna and %OM information was also collected (R^2 = 0.58 to 0.81, Table 5-5). PC1 for Farm-A (N = 90) captured slightly less of the overall variability (61%) than for Farms E and F, but still correlated well with ES (R^2 = 0.808). Hence, the relationships were considered adequate for converting the PC1 scores from the wider survey into an estimated ES value for each farm site. The predicted depositional flux for each of the farms was also converted into the same ES variable to enable direct comparisons, using the best relationships identified in Table 5-3.

The predicted area of enrichment at ES3 or greater was comparable to the observed footprints. The size of the predicted footprint at ES3 was 11.3 and 9.4 ha for Farms E and F, which compares favourably with the observed footprint 9.6 and 13.2 ha (respectively). The average total distance to the outer extent of ES3 conditions was also comparable, 102 m (predicted) and 95 m (observed) for Farm E, and 111 m (predicted) and 155 m (observed) for Farm F (Table 5-6). Both the modelled and the predicted scenarios for the dispersive farms show a generally lower and more diffuse level of enrichment. Farm-F had the widest footprint but did not exceed ES ~4.5 anywhere. These patterns are summarised in Figure 5-5a-c, which illustrates how the spatial extent increases and the impact decreases from Farm-F > Farm-E > Farm-A.

The shape and intensity of the footprints at the dispersive sites were also reasonably well predicted by the model (Figures 6 & 7). Both the model and observational data show an impacted region (ES>5) to the north-east of Farm-E (Figure 5-6); the extension of the footprint to the north-east and north-west was also evident in the model output. However, the degree of impact was slightly under-predicted by the model and the associated ES score. The predicted footprint for Farm-E was also slightly wider than the actual footprint through the centre.

The observed footprint for Farm-F was larger and more diffuse than predicted, with low level effects extending further to the south (toward the main channel) and west (Figure 5-7).

Notably the model predicted very intense effects directly beneath Farm-F which were not observed. However, overall the agreement between the observed and predicted footprints for Farm-F was good.

The agreement between the overall size of the observed and predicted footprints for the non-dispersive Farm-A was also good (Figure 5-8). Differences were on the whole were minor and mostly related to slight changes in the footprint outline. The predicted scenario had a slightly larger highly-impacted area (ES>5) directly beneath the cages, and the southern (seaward) end of the observed footprint was slightly less impacted than predicted.

Table 5-5: Best-fit linear models of PC1 in relation to ES derived from the subset of stations that were more comprehensively sampled (ES determined from empirical relationships with S^{2-}, redox, %OM, total abundance, No. Taxa, AMBI and BQI, PC1 determined from redox, S^{2-}, odour).

Farm	Equation	R^2	N
Farm-A	Y = exp(0.348x) * 2.14	0.808	17
Farm-E	Y = 0.625x + 3.125	0.720	19
Farm-F	Y = 0.651x + 2.899	0.581	18

Table 5-6: Dimensions of predicted (Pred.) and observed (Obsv.) footprints associated with two high-flow (refer Figures 6 and 7) and one low-flow (Figure 5-8) farms. Predicted footprints are based on 2010 site configurations and farming intensities.

Footprint boundary			**Farm-E** (Figure 5-6)		**Farm-F** (Figure 5-7)		**Farm-A** (Figure 5-8)	
			Pred.	**Obsv.**	**Pred.**	**Obsv.**	**Pred.**	**Obsv.**
≥ ES 3	Area (ha)	Total	11.3	9.6	9.4	13.2	7.4	6.8
	Distance (m)	Ave.	102	95	111	155	82	81
		Max.	*149*	*194*	*291*	*344*	*130*	*145*
		Min.	*31*	*38*	*40*	*62*	*20*	*15*
≥ ES 5	Area	Total	1.0	1.2	0.5	0	2.1	0.5

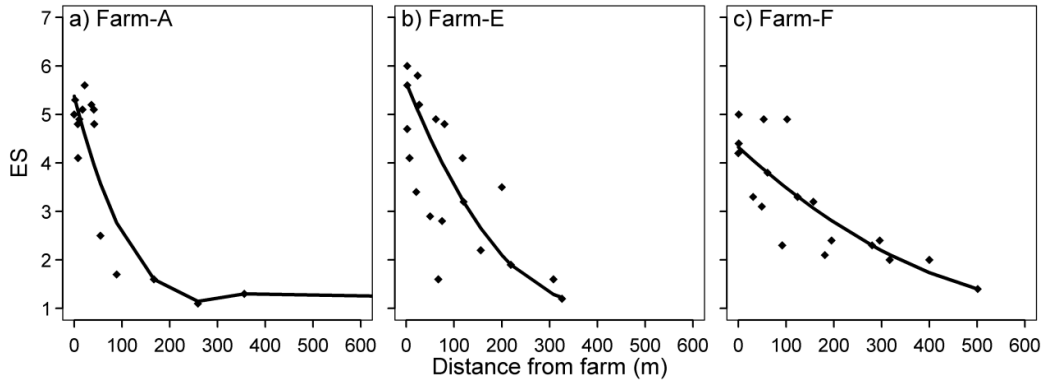


Figure 5-5a-c: Measured ES in relaton to distance from farm from the subset of stations that were more comprehensively sampled: a) Farm-A, b) Farm-E, c) Farm-F. Lines are 50th percentile quantile regression splines, where degrees of freedom (df) = 3 and degree = 2, except for Farm-A where degree = 4.

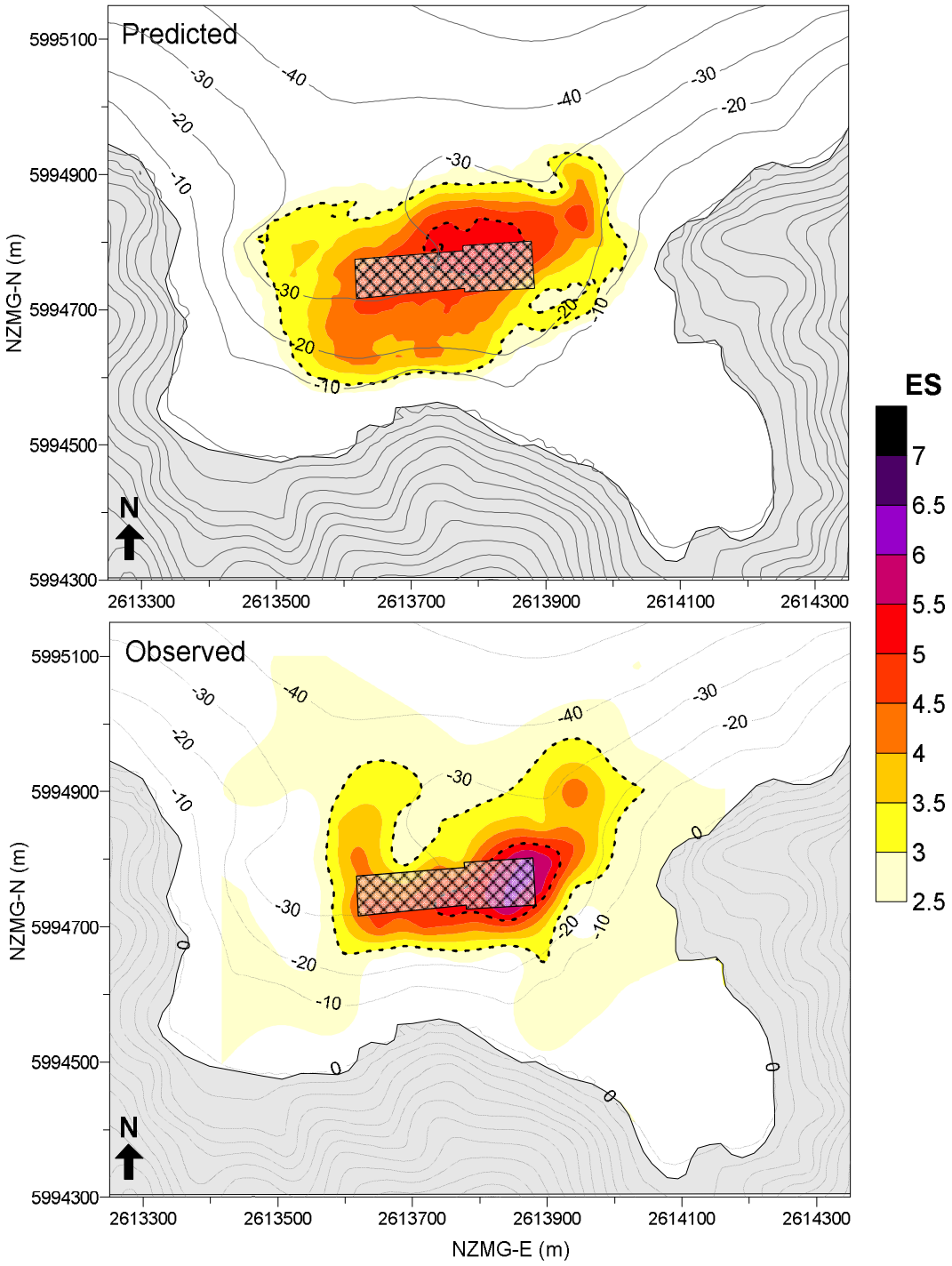


Figure 5-6: Predicted (top) and observed (bottom) benthic environmental footprints beneath the high-flow Farm-E (grey box indicates position of net pens and black dashed lines indicate ES3 and ES5 boundaries). Axes are in a national grid (NZMG), indicating distance in meters north and east of an arbitrary point south west of New Zealand. Observed ES was determined using the equations in Table 5-5. Predicted ES was determined using the regressions that were derived from the 6-month time series relationship based on depositional flux without resuspension (Table 5-3).

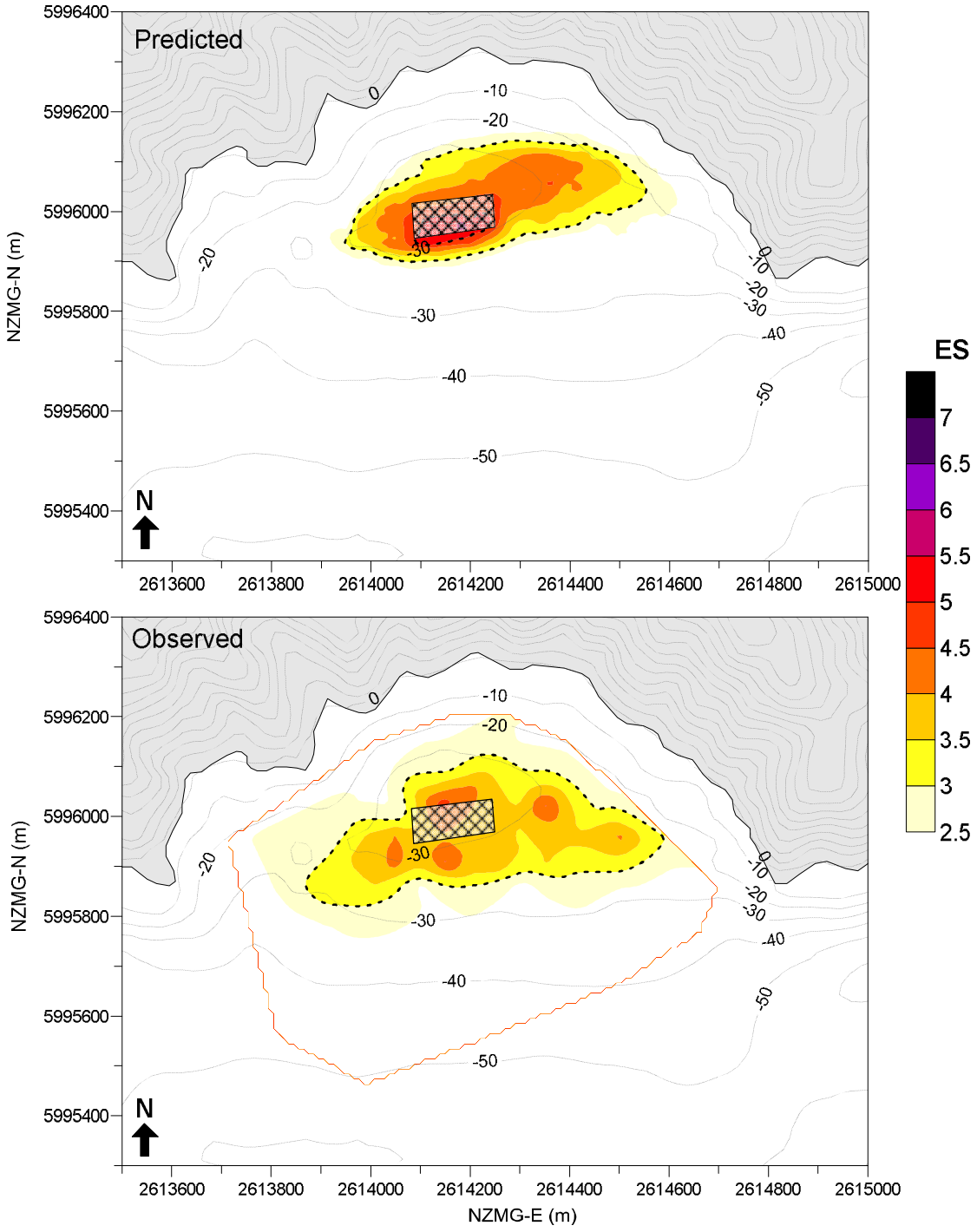


Figure 5-7: As in Figure 5-6, but for high-flow Farm-F.

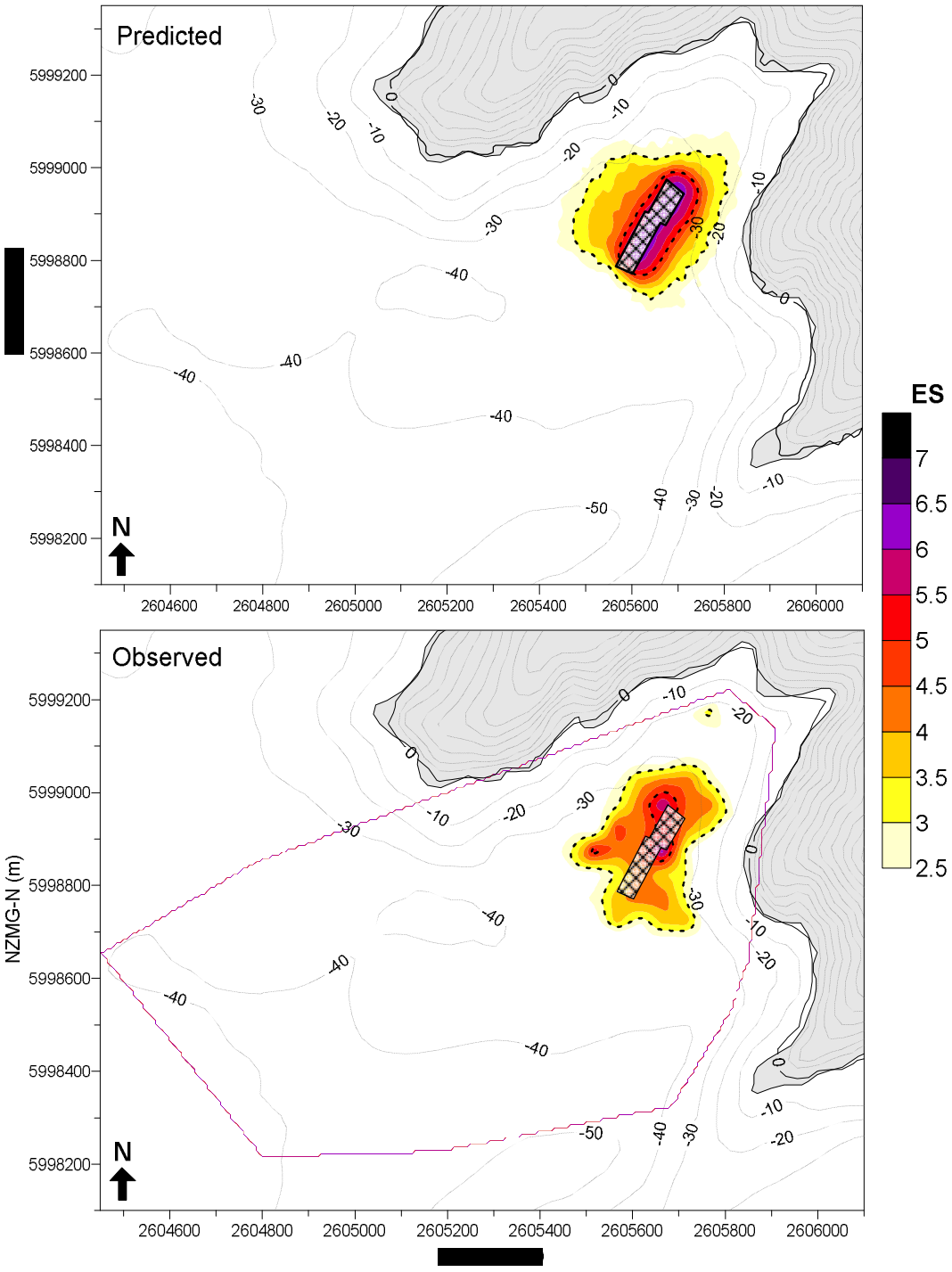


Figure 5-8: As in Figure 5-6, but for low-flow Farm-A.

5.5 Discussion

5.5.1 Predicting effects at dispersive sites

The log relationship identified between predicted flux and ES is due to large increases in enrichment in response to small increases in depositional flux over the first part of the enrichment gradient (ES1-3). Over the latter part of the enrichment gradient (ES5-7), large flux increases were associated with relatively small changes in ES. This reflects both the sensitivity to, and scope for, ecological change with the addition of organic biodeposits; this suggests that "natural" sediments will respond noticeably to small (persistent) additions of organic material, but that when sediments are already impacted, significant additions may be necessary to affect a relatively small change in enrichment stage. There are a number of possible explanations for this result. Firstly, it may be an artefact of the overall scale of change over the respective parts of the enrichment gradient (e.g., ES6 and ES7 versus ES2 and ES3) and /or, may highlight a relative insensitivity to changes in the higher enrichment stages. Alternatively, it may reflect the fact that the impact gradient is bounded and that conditions cannot get appreciably worse than those indicated by ~ES6.5, and therefore there is limited scope for further degradation with any additional feed inputs. The mostly likely scenario is a combination of these two mechanisms, whereby the degree of change indicated by the macrofauna-related variables at that end of the spectrum is limited (Keeley et al. 2012a) and bounds our present understanding as to the limits of effects. The additional capacity is presumably facilitated by the seabed progressing from an assimilative phase, where the macrofauna are prolific, to a state of organic accumulation, dominated by microbial processes and where changes may be better defined by other physico-chemical type variables.

When the process of resuspension was modelled at the two dispersive sites predictions indicated that all particles would be exported, irrespective of the critical resuspension velocity used (i.e., vr = 9.5, 12 or 15 cm s^{-1}). According to the conventionally held view that benthic effects are proportional to depositional flux (Cromey et al. 2002a), the resultant effects would be negligible - but this was not the case. There was minimal evidence of organic accumulation (indicated by %OM), however, pronounced ecological effects were identified at both dispersive sites. A similar observation was made by (Chamberlain & Stucchi 2007) at a moderately dispersive site in Canada, where DEPOMOD predicted that virtually all of the material would be exported from the site, yet localized seabed enrichment was evident. This suggests that

either the resuspension component of the model is over predicting how much material is being exported, or the model is correct and the popular understanding of how ecological effects are induced at dispersive sites is incomplete.

Over-prediction of particle advection by the model may occur where the critical resuspension velocity (vr) is set too low, or where the numerical algorithms describing resuspension do not consistently represent the key dynamical processes. Chamberlain and Stucchi (2007) suggested that the default vr in DEPOMOD (9.5 cm s^{-1}, previously 'hard-coded' into the model) may indeed be too low, but also that using a single value was probably too simplistic, given the difference between the vr's required to suspend feed pellets compared with fish faeces. The current study showed that observed effects occurred in conjunction with a predicted flux of zero when a vr of 9.5 cm s^{-1} was used, but that this disparity decreased as vr was increased toward 15 cm s^{-1} – thereby decreasing predictions of total advection. By experimenting with even higher vr values at the two dispersive sites (model outputs not shown), it was determined that vr values in excess of 35 cm s^{-1} would be required in order for significant accumulation to occur. Waste feed pellets are known to roll and saltate (bounce) at current speeds of 16 - 20 and 32 - 40 cm s^{-1}, respectively (Sutherland et al. 2006), consequently it is likely that the resuspension of those particles was over-predicted by the model. However, at the sites in this study, waste feed was recently estimated to be < 1 % (Cairney & Morrisey 2011), and therefore the deposition would have comprised mostly faecal particles, which resuspend at much lower current speeds - in the order of 7 to 15 cm s^{-1} (Cromey et al. 2002b). Given that the physical properties of the main biodeposits (i.e. feed pellets or faeces) would be broadly comparable irrespective of region and/ or site characteristics, the vr values that would be required to achieve particle accumulation at the dispersive sites seem unrealistically high. Hence, it seems more likely that the model predictions using the default vr setting are reasonably accurate and that the observed impacts are occurring in the absence of significant organic accumulation. This effect has been described in these dispersive systems (Keeley et al. 2013a) and is characterised by proliferation of opportunistic taxa in the presence of an elevated carbon flux and a strong oxygen supply, but in the absence of significant organic accumulation and the associated sediment anoxia, which would normally limit biological production (Findlay & Watling 1997, Hargrave et al. 2008).

Although the model outputs incorporating resuspension may be faithfully reproducing the physical processes, the results are not very useful for the purposes of predicting either the

spatial extent or magnitude of seabed effects at higher flow sites. Using the no-resuspension scenarios to predict flow-specific effects, in a similar manner to that adopted by Chamberlain and Stucchi (2007), we established separate relationships between predicted flux and overall enrichment effects (ES) for non-dispersive and dispersive sites; the main difference being that a greater discharge was required to induce an equivalent level of effects at the dispersive sites. According to these relationships, moderate, detectable levels of enrichment (i.e. ES3) occur with the addition of approximately 0.4 kg solids m^{-2} yr^{-1} for non-dispersive sites and ~1 kg solids m^{-2} yr^{-1} for dispersive sites. ES5-type impacts, indicative of peak abundance beyond which the macrofauna is at increased risk of a collapse (ES6-7, Keeley et al. 2012a), are induced by the addition of ~6 kg m^{-2} yr^{-1} for non-dispersive sites and approximately double that amount for dispersive sites (i.e. ~13 kg m^{-2} yr^{-1}). The difference between these two thresholds (i.e. ~5 kg m^{-2} yr^{-1} or ~50%), which compare favourably with previous attempts to link depositional flux to enrichment response (Table 5-7), may be related to the amount of material that is being exported from the immediate vicinity, over and above what is either settling (and being buried) or being biologically assimilated locally.

A flux rate, over and above natural background sedimentation, of around 1 - 1.5 kg m^{-2} yr^{-1} has been identified in several previous studies as the point at which clear changes in the macrofauna community and/ or the oxic status of soft sediments may be observed (Hargrave 1994, Findlay & Watling 1997, Cromey et al. 2002a, Chamberlain & Stucchi 2007, Cromey et al. 2012). These estimates are slightly higher than those identified for ES3 at non-dispersive sites in the present study (i.e. ~0.4 kg m^{-2} yr^{-1}). However, it is difficult to determine the exact level of enrichment referred to in each case due to the differing suites of individual indicators and threshold descriptions that are employed. Accordingly, it is possible that the enrichment level (ES3) used in the present study, based on multiple indictors, represent a more sensitive threshold. The particular ecosystem effect to be assessed may also influence the required sensitivity of the measured response. For instance, Holmer et al. (2008) identified a similar flux (0.5 kg m^{-2} yr^{-1}) as the point beyond which seagrass shoot mortality was accelerated, whilst the suggested threshold for effects to more sensitive Maerl bed communities would appear to be appreciably lower at 0.1 kg m^{-2} yr^{-1} (Sanz-Lazaro et al. 2011). Cromey et al. (2002a) associated the peak in opportunistic taxa, which equates to ES5-type conditions, with a depositional flux of 10 kg m^{-2} yr^{-1} for non-dispersive sites, which is double that proposed for comparable flow regimes in this study (4-5 kg solids m^{-2} yr^{-1}) and still less than the estimate for dispersive sites. In addition, some of the minor variances between studies may be due to regional

environmental specificity and differences in the quality of the particles being deposited (i.e. feed waste presumably has higher enrichment potential than faecal waste, Chamberlain & Stucchi 2007).

For the purposes of this study, sites were categorised as being either dispersive or non-dispersive based on their current speeds and how these relate to the vr of 9.5 cm s^{-1}. Sites with near-bottom speeds above vr greater than 50% of time were treated as 'dispersive'; this categorisation was both conceptually logical and consistent with observations of how the seabed effects manifested at the sites over the previous 10 years. Sites with 'intermediate' physical properties (central to this threshold), or with notably higher current speeds, may require special consideration (e.g. use of an alternative flux – ES relationship).

Relationships between predicted flux and individual indicator variables were generally weaker than those with ES, which integrates multiple biotic and abiotic variables. Of the individual indicators AMBI appeared most versatile, relating to flux at both non-dispersive and dispersive sites. This result is not surprising given that the AMBI is considered to be a good predictor of overall enrichment state (Keeley et al. 2012a). Macrofauna abundance (N) was particularly poorly predicted by flux at non-dispersive sites, being highly variable when flux was elevated. However, there was a notable spike in N at both the dispersive and non-dispersive sites at around 10 kg m^{-2} yr^{-1}, which aligns reasonably well with both the position of the abundance and biomass peaks identified by Cromey et al. (2012), and ES5 conditions, as described above. Species richness (S) was strongly negatively correlated with flux at the non-dispersive sites, which was consistent with Cromey et al. (2012), who observed a relatively consistent decline below ~0.1 kg m^{-2} yr^{-1}. Whereas S showed a relatively poor relationship with flux at the dispersive sites, presumably because high flow environments tend to be more resilient to deposition (Keeley et al. 2013a). This observation is symptomatic of the processes discussed above, whereby the seabed encounters high levels of depositional flux, but as much of it is exported, accumulation and the associated physico-chemical effects are limited.

What we appear to be observing is a an effect that may be relatively unique in nature but common in fish farming, associated with the continual rain of organic material, which results in a situation where the sediment is enriched even though there is no net accumulation because material is constantly being advected away. Therefore, using non-resuspension scenarios to predict effects for such high flow sites is appropriate on the basis that it represents the

'primary footprint', defined as where the particles may fall on initial settlement and where effects are most pronounced. Subsequently, particles from resuspension and horizontal transportation that may result in alterations to the overall size or shape of the footprint, but that this would only result if the loss from erosion at the outer margin of effects, and from particles going into solution and being assimilated by the environment, is less than the load that is being redistributed. However, this process was encompassed to some extent in this study, as most of the sites have been consistently utilised for many (>5) years and therefore should be in a relatively stable state.

Using the primary footprint to gauge the extent of the 'main effects' for new or proposed sites can provide useful guidance for setting initial farm management objectives (e.g., allowable zone of effect's, AZE). On this issue, it is important to recognise that the present ASC standards (ASC 2012) for the AZE for salmon farming permit a relatively modified state, whilst the discussion provided above considers less obvious potential effects beyond that zone. Effects in the outer regions will be inherently subtle and difficult to definitively distinguish from 'natural' change. Consequently, delineating a more accurate 'impacts' boundary will always be challenging and fraught with subjectivity.

Table 5-7: Summary of proposed depositional flux thresholds and the associated benthic enrichment effects.

Source:	Depositional flux gC m^{-2} d^{-1}	Depositional flux kg solids m^{-2} yr^{-1}	Associated ecological threshold/conditions	
This study: (average values)	**0.28**	**0.35**	Non-dispersive sites	**ES3 (Moderate/ detectable enrichment)**
	0.76	**0.93**	Dispersive sites	
	4.9	**5.9**	Non-dispersive sites	**ES5 (Highly enriched)**
	11.2	**13.6**	Dispersive sites	
Dahlbäck and Gunnarsson (1981)	1.7	2.1	Enriched seabed beneath blue mussel farms.	
Hargrave (1994)	1	1.2	Formation of hypoxic sediments around salmon farms.	
Findlay and Watling (1997)	1 to 5	1.2 to 6.1	Threshold at which macrofauna biodiversity reduced by salmon biodeposits.	
Cromey et al. (2002a)	0.01	0.01	Macrofauna change begins based on ITI.	
	0.82	1	Significant change in composition.	
	8.22	10	Corresponds to peak in opportunists.	
Chamberlain and Stucchi (2007)	1 to 5	1.2 to 6.1	Significant change in macrofauna community (also transition between oxic/ healthy and anoxic/ degraded benthic zonation status).	
(Weise et al. 2009)	>4.5	>5.5	Significant alterations to the benthic community beneath mussel farms	
Sanz-Lazaro et al. (2011)	0.087	0.1	To maintain diversity of Maerl beds.	
Cromey et al. (2012)	1.23	1.50	Boundary beyond which clear pollution indicative changes occur in macrofauna.	

Using shorter feed time-series made very little difference to the robustness of the relationships between predicted and observed effects, suggesting that there is little to be gained in terms of resolving temporal dynamics in enrichment effects from using higher temporal resolution feed information, especially if it is in the absence of finer resolution, cage-scale stocking/ feed use information. Therefore, using the average feed consumption information for the medium-term (c. 3 or 6 month) period preceding the required benthic evaluation appears to be adequate for predicting effects.

In both the dispersive and non-dispersive examples, there was some scatter about the data. This may in part be related to minor inaccuracies with recreating the spatial arrangements in the models (i.e. positioning the sample stations in relation to the farms), and/ or the inability to accurately recreate historical farming conditions. For example, it was not possible to include within-farm stocking variations (i.e. temporarily empty nets and fish rotation). Additionally, the application of a constant waste feed value (which has a strong influence on flux estimates, Chamberlain & Stucchi 2007) was probably overly simplistic as

improvements in feeding techniques are likely to have reduced wastage over the study period. Finally, some of the scatter may also be due to natural spatial and temporal variability in the benthos (e.g., Thrush 1991), which in-turn may be more pronounced under highly enriched conditions. Nevertheless, the errors presumably operated in both directions (over and under estimation) the measures of central tendency described should remain valid.

5.5.2 Spatial comparison of predicted and observed footprints

Overall, the predicted footprints using the no-resuspension scenarios corresponded well to the observed footprint in terms of size, shape and overall intensity. Hence, the use of non-resuspension scenarios to predict the effects at dispersive sites appears valid, particularly when ES3, indicative of moderate/ detectable enrichment, is used to delineate the outer extent of effects. The ES3 threshold was selected because it clearly indicates anthropogenic enrichment; ES levels < 3 can occur naturally (Keeley et al. 2012a). Using thresholds <ES3 increases the risk of including areas that are not necessarily enriched as a result of farm activities in the footprint. ES≥3.0 is therefore recommended as a useful limit for delineating farm effects boundaries unless there are good grounds to justify a lower threshold, i.e. comprehensive baseline information.

Agreement between the predicted and observed footprints declined in the more severely impacted regions (i.e. directly beneath the cages). This may be due to the lack of observational data from directly beneath the cages and / or to an overestimation of feed wastage. Although severe impacts might be expected at non-dispersive sites, this would be less likely at dispersive sites where strong currents can diffuse the intensity of impact. A recent study conducted at Farm-F showed that feed wastage was < 1 % (Cairney & Morrisey 2011). The modelling in the present study was conducted with a feed waste of 3% for the reasons outlined in the methods. Chamberlain and Stucchi (2007) suggest that waste feed is responsible for the majority (i.e. 70% at 5% waste) of the carbon flux beneath the cages and as far as 60 m away, but beyond that the contribution is dominated by the smaller and more slowly settling faecal particles. Therefore, if the farms can achieve near-zero feed wastage, then the impacts under and near to the cages may be reduced. The effect of using a 1 % waste feed level was tested for Farm-F, with the results indicating that the footprint (ES>3) was a similar shape and size (0.2 % smaller), but that the area of seabed predicted to be impacted to ES>5 was slightly smaller (by 0.26 ha, or 2.3 % of the footprint). As such, the effect of adjusting the waste parameter by 2 % for the given scenarios was assessed to be minor. In addition,

some of the shape aberrations may reflect fine-scale farm use practices (e.g. periodically empty nets within farms and/ or any temporary extensions or contractions of farms) or hydrodynamic conditions (e.g. storm events) that were not captured by the models.

5.6 Conclusion

Localised benthic impacts may still be observed even where depositional models suggest otherwise, as significant benthic effects can occur in the perceived absence of organic 'accumulation'. A useful indication of the spatial extent of such effects can be obtained when the model is parameterised without resuspension: this suggests that approximately twice the amount of deposition flux is required to induce effects at dispersive sites compared to non-dispersive sites. Specifically, moderately enriched conditions (ES3) were associated with approximately ~0.4 kg m^{-2} yr^{-1} for non-dispersive sites and ~1 kg m^{-2} yr^{-1} for dispersive sites and highly enriched conditions, (peak infauna abundance - ES5), with approximately 6 kg m^{-2} yr^{-1} and 13 kg m^{-2} yr^{-1} for non-dispersive and dispersive sites, respectively.

Three main interactive ecosystem process components underpin the ultimate enrichment response (Figure 5-9). At non-dispersive sites, total deposition (A) almost entirely equates to net deposition (B), which comprises B1 (settlement, consolidation and ultimately burial) and B2 (assimilation by benthic biota), with little or no influence from C (resuspension). In contrast, at dispersive sites, B1 is minimal and the impact is characterised by processes B2 and C1 (water column dilution and assimilation by biota) with the additional influence of far field deposition and subsequent assimilation and burial (C2); these processes together comprise the resuspension and advection process (C).

Where there is a large footprint (i.e. dispersive sites), in combination with significant sediment resuspension and advection (process C) and abundant opportunistic taxa (i.e. a larger B2 component) then the overall load to the ecosystem (A) can be much larger: in this study the seabed at the dispersive sites sustained twice as much particulate flux as the non-dispersive sites. Whilst the ratio between B and C was not quantified in this study, the differences between the flux required to induce equivalent levels of effects at the disspersive and non-dispersive sites provides some indication of this response, i.e. ~ 7 kg m^{2} yr^{-1} at ES5, or ~50% of A. Understanding the empirical relationship between C1 and C2 is particularly

important for characterising impacts at dispersive sites and would be a worthwhile area for further research.

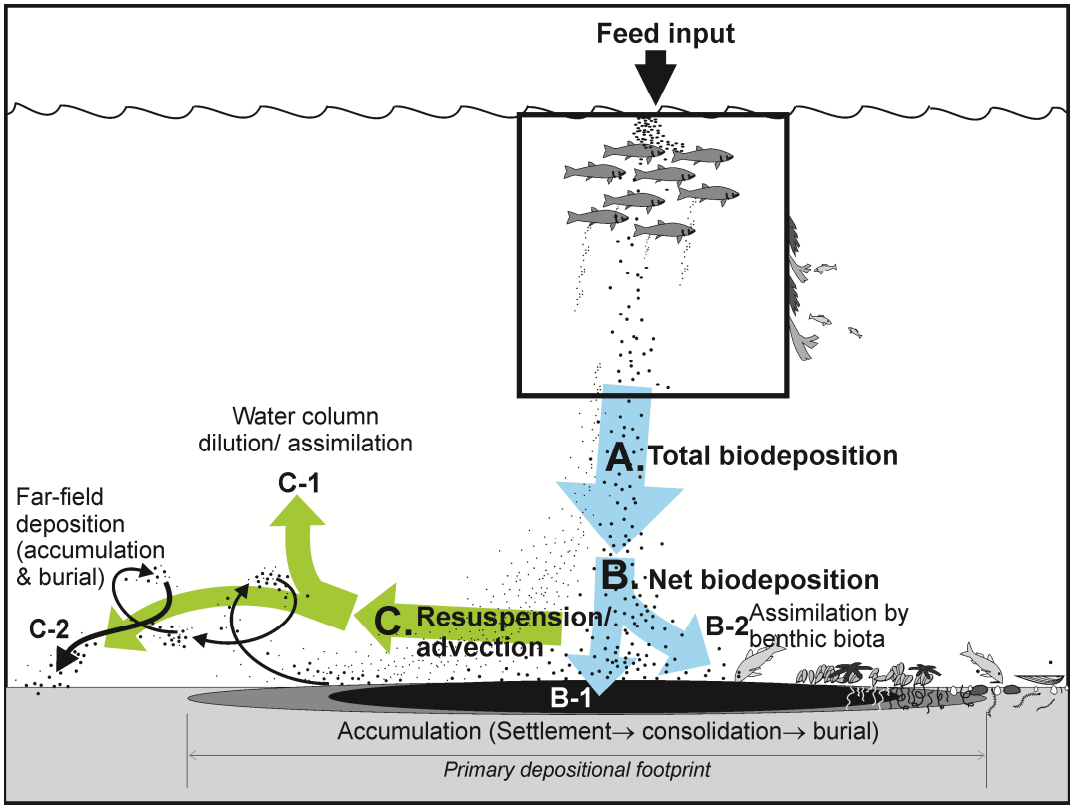


Figure 5-9: Summary of major pathways for salmon farm feed-derived biodeposition, A. Total biodeposition = all waste particulates produced from farm (feed and faeces - ignoring dissolved organic component), B. Net biodeposition is the particulates that settle, accumulate and / or are used (assimilated) in the near-field or 'primary footprint', C. Resuspension and advection includes the fraction of A that is exported from the immediate vicinity by currents.

CHAPTER 6

SPATIAL AND TEMPORAL DYNAMICS IN MACROBENTHOS DURING RECOVERY FROM SALMON FARM INDUCED ORGANIC ENRICHMENT: WHEN IS RECOVERY COMPLETE?

Preface:

This chapter utilises a 10 year dataset detailing recovery from a highly impacted state at a low flow site. The dataset enabled spatial and temporal patterns to be explored and a potential recovery 'end-point' to be identified. In doing so, it also provides a framework for evaluating different definitions and metrics of recovery, as well as some fundamental ecological concepts (e.g. the role of key taxa in remediation, and ecological succession end-points).

At the time of thesis submission, this work had been accepted for publication in a peer-reviewed journal subject to minor revisions. The manuscript has been adjusted to a standard format for the thesis, and as such there may be minor differences in the text, figures and tables compared with the published version. The citation for the original publication is:

Keeley NB, Macleod CK, Hopkins GA, Forrest BM. In Review. Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: when is recovery complete? Submitted on MPB on 07/08/13 and accepted subject to minor revisions on 02/09/13.

6.1 Abstract

This study analyses a temporal dataset documenting 8 years of benthic recovery at a highly impacted salmon farm. Quantifying the endpoint of 'recovery' proved challenging due to: lack of a widely accepted definition, dynamic spatial and temporal equilibria, inherent variability in recovering sediments, differing trajectories of impact and reference sites, and statistical challenges. More complex biotic indices and metrics incorporating multiple variables were most robust. Tests for 'parallelism' proved useful, but results were contingent upon how they were applied and should therefore be used in conjunction with data-visualisation methods. Substantial recovery occurred in the first 2 years, and was assessed to be complete after ~5 years. However, minor differences were still evident along with some on-going benthic instability, attributable to medium-scale spatial movements and successional patterns of macrobenthos. The study highlights the importance of having a predetermined recovery endpoint, and using multiple indicators and a weight-of-evidence assessment approach.

6.2 Introduction

In marine benthic systems, impacts associated with organic enrichment are common and widespread, due to the prevalence of diffuse (e.g. land runoff, Diaz & Rosenberg 2011) and point source (e.g. outfalls, Taylor et al. 1998, Cardell et al. 1999) discharges of anthropogenic wastes. Two considerations that are critical to evaluating the degree of impact on the environment are spatial scale and 'reversibility' of effects. Strong gradients of ecological succession are common, and the fundamental biological and chemical changes are generally well described (Pearson & Rosenberg 1978, Gray et al. 1979, Kalantzi & Karakassis 2006). However, there is less certainty associated with delineating the outer extent of enrichment effects, mainly due to natural variability (in both time and space) in environmental condition (e.g. Thrush 1991, Hewitt et al. 1997, Hewit & Thrush 2007) and often a lack of understanding around what constitutes 'natural' conditions.

Finfish aquaculture is a significant point source of organic matter (via waste feed and fish faeces) to the marine environment, and provides a good case study for understanding benthic enrichment processes (Keeley et al. 2012a). The primary discharges of waste feed and faeces normally results in highly enriched conditions in the immediate vicinity of the farm (Karakassis et al. 2000, Brooks et al. 2002). In extreme cases, conditions immediately beneath the stocked cages can become anoxic, and virtually azoic (no animal life present), in which case the pathway to recovery will be maximised in terms of the enrichment/ disturbance gradient (Pearson & Rosenberg 1978, Keeley et al. 2012a). However, a wide range of farming conditions can be encountered in aquaculture (i.e. farm type, intensity and age) providing a variety of impact start points (e.g. Borja et al. 2009). The practice of site fallowing (temporarily retiring a site) that is often used for management purposes also provides commercial incentive for understanding the recovery process.

Estimates of benthic recovery times vary greatly, ranging from weeks (Ritz et al. 1989) to >11 years (Wan Hussin et al. 2012). Several studies, especially those undertaken around smaller fish farms, have suggested that complete recovery (biological and chemical) can occur within 6 months of fallowing (Brooks et al. 2003), and in some cases within periods as short as 7 to 14 weeks (Ritz et al. 1989, Brooks, 2003, cited in Brooks et al. 2004). The general consensus from studies conducted over the medium-term (i.e. up to 3 years), is that marked improvement occurs in the first 6 to 12 months, but that recovery generally remained

incomplete (Karakassis et al. 1999, Pereira et al. 2004, Lin & Bailey-Brock 2008, Macleod et al. 2008, Villnas et al. 2011). Long-term (i.e. > 3 years) studies of recovery are scarce; one that was conducted over 7 years estimated full chemical remediation would take 5.3 years and that biological remediation may take much longer (Brooks et al. 2004).

While the spread of these estimates may be partly attributable to the levels of impact at the point of fallowing and varying underlying environmental conditions, there are also multiple definitions of recovery that may be contributing to the variances. Brooks et al. (2003) distinguished biological and chemical remediation; highlighting characteristically different pathways, and providing specific criteria for 'recovery' in each case. Other studies have emphasised differences between species-based, community recovery and 'functional recovery' (Macleod et al. 2008); i.e. the point at which ecosystem function is re-established, but not necessarily with the same communities that were present pre-impact. It is generally assumed, that once functional recovery is achieved, an "equilibrium state" will ensue (Young et al. 2001, Macleod et al. 2008). The concept of 'sustainable ecological succession', indicated by consistent presence and abundances of a limited number of species, has also been proposed as a good measure of recovery (Ellis 2003).

The difficulties associated with determining the point of 'recovery' are further exacerbated by problems that arise when attempting to evaluate the question statistically. Many impact studies lack an appropriately defined assessment of pre-impact conditions, against which recovery can be quantitatively compared. Consequently, recovery is assessed by comparison of conditions against selected spatial reference sites, that may in fact be naturally different, and the opportunity to evaluate the degree of change at a particular site is lost. Another problem with using spatial comparison as the reference point for recovery is that it may not always be appropriate to assume a strict equilibrium (or a single 'stable state') in biological systems (Beisner et al. 2003, Parker & Wiens 2005). There may instead be a 'dynamic equilibrium' (Parker & Wiens 2005, Macleod et al. 2008) and / or several possible alternative stable states (Beisner et al. 2003). Hence recovery should be assessed against a backdrop of both temporal and spatial variation.

Conventional beyond-BACI designs (e.g., Underwood 1991, 1992) go some way to achieving this. However, they tend to be resource intensive, requiring both multiple reference sites, and multiple randomly timed samplings within each specified time window. Few multi-

year monitoring programs are initiated with this level of sampling effort in place, and maintaining such a design over a long timeframe is unlikely as the cost can be prohibitive. In addition, although beyond-BACI designs clearly partition the multiple sources of variation, the design is premised upon there being two fixed periods, 'before' and 'after' (e.g., Aguado-Giménez et al. 2012), whereas in most long-term datasets time is often a continuous variable that may reveal a non-linear response. Therefore, with a beyond-BACI approach it can be difficult to directly address the questions "was recovery complete?" and if so, "when did it occur?"

Recovery can be conceptually defined as occurring when the impacted (injured) resource reaches the level which it would have been, had it not been impacted in the first place. At which point, the influence of impact-related factors will have diminished to the point where levels of the resource vary temporally in a natural way (Parker & Wiens 2005 and U.S. Code of Federal Regulation, 2001). The concept of 'varying temporally in a natural' way implies an assumption of 'parallelism', whereby impact and reference sites will begin to respond similarly to, for example, wider climatic influences. This is useful statistically, and methods (based on the BACI approach) have been developed accordingly, and used to assess recovery from the *Exxon Valdez* oil spill (Skalski et al. 2001). These methods appear to have broader applications.

Here we provide an analysis of a 10 year annual monitoring program documenting recovery over the last 8 years from a highly impacted state. A variety of existing and novel methods are used to evaluate the remediation process and explore the concept of recovery 'end points'. In doing, so we identify key stages and ecological indicators of the recovery process.

6.3 Methods

6.3.1 Study sites and sampling procedures

This study was conducted at a commercial Chinook salmon (*Oncorhynchus tshawytscha*) farm site located in the outer reaches of the Marlborough Sounds, New Zealand (Figure 6-1). The farm was situated in a sheltered embayment over muddy-sand sediments (average mud

content = 78 to 84%), in water depths ranging between 28 and 35 m, with relatively low current speeds (mid-water mean current speed ≈ 3 cm s^{-1}).

The farm was fallowed in 2001 after approximately seven years of consistent and relatively intensive use (average feed usage of ~180 mt $month^{-1}$). Benthic sampling was undertaken in the Austral spring (October/November) as follows: two years prior to fallowing (T-2), immediately after the farm was fallowed in 2001 (T0) up until 2009 (T8). No sampling was undertaken in 2000 and 2008. Seabed samples were collected at 25 m intervals along a north-western transect (Gradient stations) running away from the farm ('Cage stations'), and at fixed Reference stations (Figure 6-1). Not all sampling stations were sampled in every year; most notably, two further reference sites were added at ~400 m and ~5800 m in 2003 and 2009 respectively (sampling events denoted by '×' on subplots in Figure 6-2).

At each station, sediment samples were collected using a van-Veen grab, with water depth (Depth, m) and distance from farm (Distance, m) recorded. All samples were collected in triplicate (i.e. n=3), except in 2006 (n=2) and 2009 (n=5). Sediments were retained from each sample for the determination of grain size distribution (dried and analysed gravimetrically for size class fractions from silt-clay through to gravel), organic matter content (%OM measured as % ash free dry weight; Luczak et al. 1997) and macrofaunal community composition. Sediment grain size and %OM measures were determined from sub-samples collected using a 5.5 cm diameter Perspex core, with the surface 30 mm kept for analysis. Macrofauna sub-samples were collected using a 130 mm diameter (0.0132 m^2) core (100 mm sediment depth). Macrofauna were sorted and enumerated to the lowest practicable level and their abundances recorded. Macrofauna count data were used to calculate total abundance (N), number of taxa (S), Pielou's *(J')*, Shannon *(H')* and the AZTI's Marine Biotic Index (AMBI, Borja et al. 2000), Benthic Quality Index (BQI, Rosenberg et al. 2004) and Multivariate-AMBI (M-AMBI, Muxika et al. 2007). Qualitative assessments of sediment odour, Beggiatoa (bacterial mat) coverage and sediment out-gassing using pre-specified categories (as described in Keeley et al. 2012b) were also made at each station.

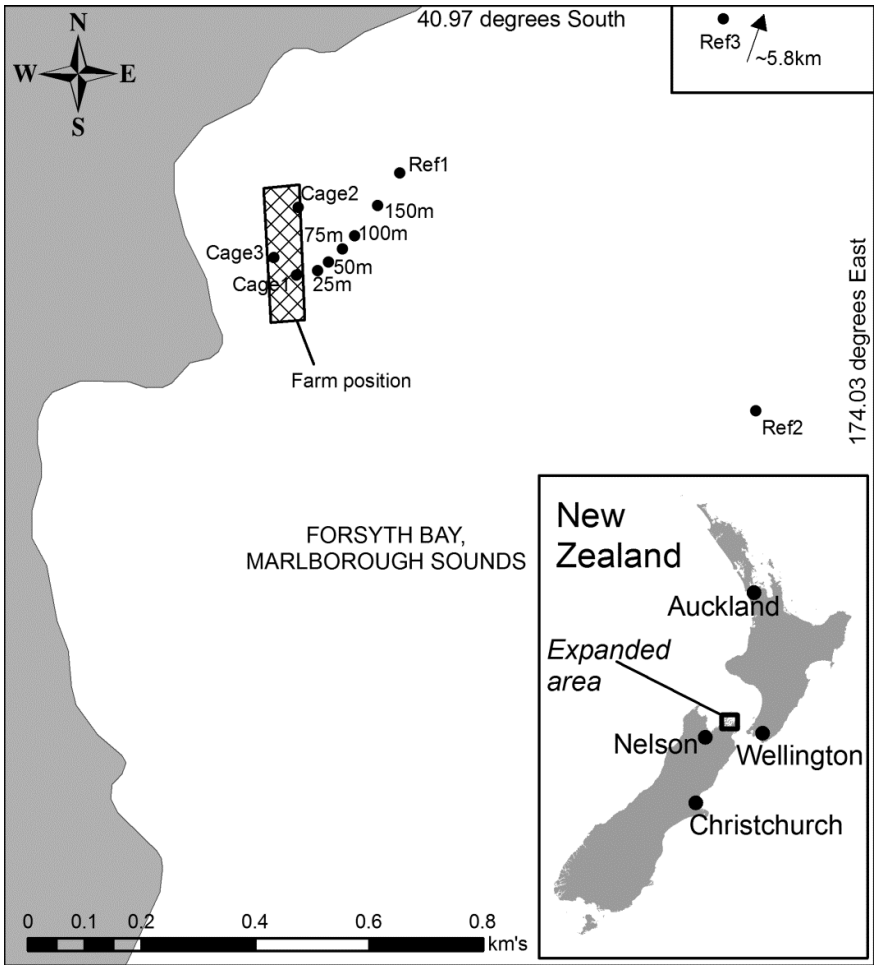


Figure 6-1: Location of study site and sampling stations in relation to the farm

6.4 Data analyses

6.4.1 Indicators of recovery

Pearson correlation coefficients between variables were determined from replicate-level data, with a $\log_{10}$ transformation applied to strongly right-skewed variables (i.e., N). Several of the environmental indicators were highly correlated in space and time. A subset of variables was selected for further analysis: based on their potential to provide complementary information, due to weak correlations, representing different components of the benthos and / or different levels of analytical complexities (e.g. biotic indices). These included: %OM, $\log_{10}$(N), S, AMBI, and BQI, as well as overall enrichment stage (ES). ES is a numerical derivative of all of the physico-chemical and biological variables combined, and therefore provides a robust indication of overall impact status (Keeley et al. 2012a). Although BQI and AMBI were highly correlated, both were retained because they have previously been found to be particularly good indicators of enrichment (Keeley et al. 2012a and references therein)and are computationally quite different and utilise different taxa pollution tolerance classification schemes (Borja et al. 2000, Rosenberg et al. 2004). Furthermore, there are defined values for BQI and AMBI which specifically relate to levels of benthic quality and pollution status (Borja et al. 2000, Rosenberg et al. 2004); these classifications are shown in Figure 6-2.

Spatial and temporal relationships for individual parameters were interpolated into a grid using the Kriging method in Surfer 9, and displayed in 2-dimensional plots. The x-axis represents ‘Time’ in years relative to fallowing (T0) and the y-axis represents ‘Space’ as distance from cages (m).

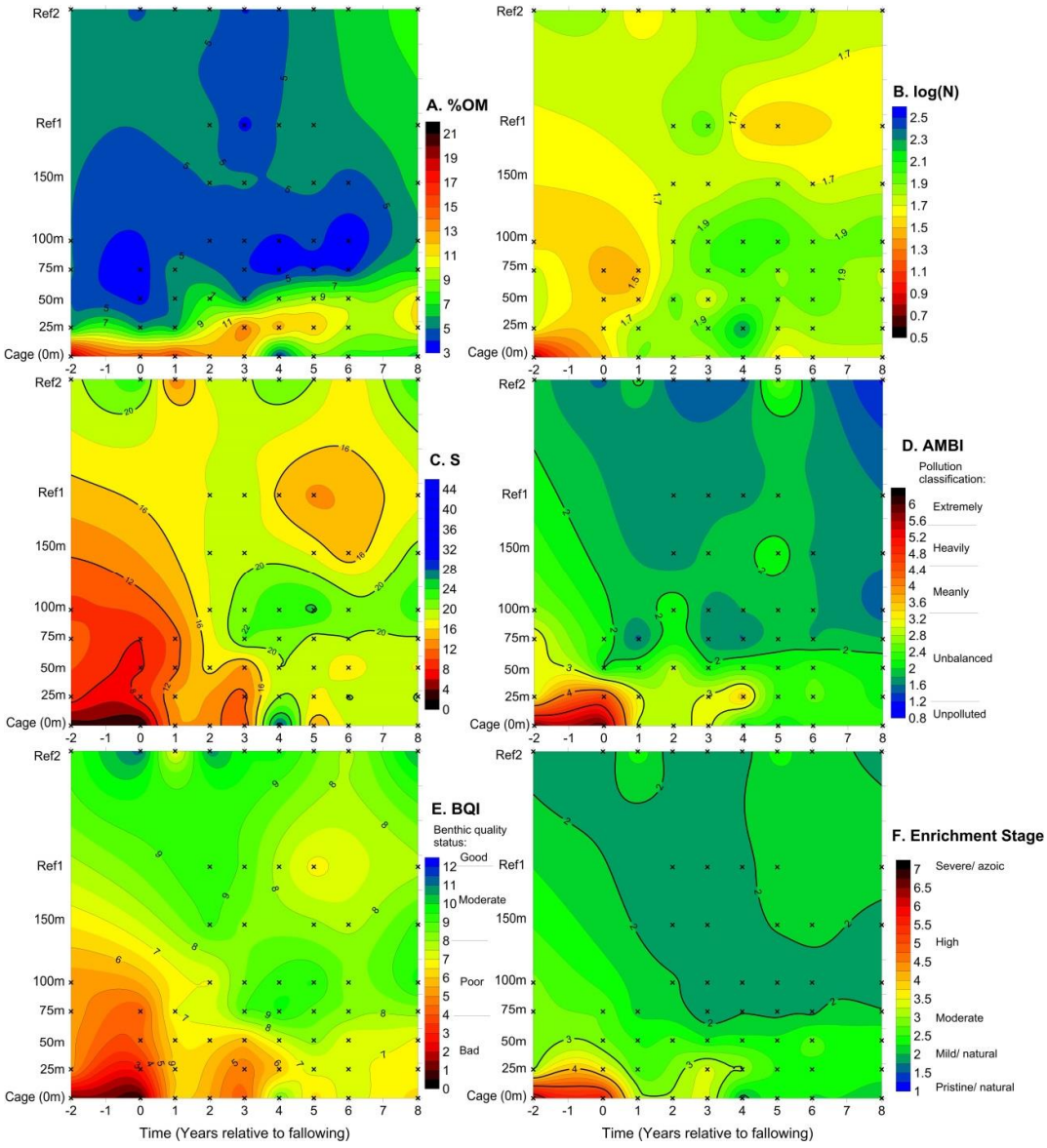


Figure 6-2: 2-dimensional plot of changes in %OM and log(N+1), S, AMBI, BQI and ES with space and time at for the study site. Time is given in years relative to year of fallowing (= T0) and space represents distance (m) from the cages. AMBI index – high values indicate a more polluted status (Borja, 2000); BQI index - high values indicate the high benthic quality (Rosenberg, 2004); ES - lower values reflect lower levels of enrichment (Keeley et al., 2012a). Crosses denote sampling events.

6.4.2 Multivariate analysis

Macrofaunal community data were analysed using PRIMER 6 (Clarke 2006). Data were square-root transformed to reduce the influence of the highly abundant taxa and then averaged at the station-level. Multi-dimensional scaling (MDS) was used to display the Bray-Curtis similarities (zero adjusted with dummy variable due to some samples containing very few individuals, Clarke et al. 2006) between Cage, 25 m, 75 m and Reference stations over time (all years: T-2 to T8). One-way, single factor ANOSIM (Clarke 2006) was also conducted on replicate-level data to obtain a pair-wise assessment of statistical differences at each point of survey (i.e. T-2 to T8).

Species succession was described by using the SIMPER procedure to identify those taxa which contributed most to Bray-Curtis similarities within sample groups for each survey; groups consisted of sample replicates pooled within Cage or References stations. Bubble plots were generated to display temporal patterns in specific taxa using the ggplot() function in the ggplot2 library in R, where the x-axis represents time (in years or months) and the y-axis represents individual species. The colour gradient of the symbols indicates the average (%) contribution to the groups' similarity (from SIMPER analysis), and bubble size indicates abundance (square-root transformed) at the given time. Taxa shown are restricted to those which contributed to the top 90% of the dissimilarity. The y-axis (i.e. taxa) was sorted (from top to bottom) according to the sequential contribution of those taxa to the groups' similarity, starting with T0 and progressing to T8. This creates a gradient of species succession; with those species that played an important role early in each study (i.e. either immediately post-fallowing or post farm re-introduction) placed at the top of the plot and those that contributed in the later stages (e.g. recolonization) toward the bottom. Taxa-specific 'Eco-Groups' (EG) are displayed where available, which are established classifications for species sensitivity to organic enrichment that are used to calculate AMBI; these groups range from 'I' (very sensitive) to 'V' (first order opportunistic taxa) (refer Borja et al. 2000, Keeley et al. 2012b and http://ambi.azti.es/).

Macrofauna data were also analysed according to functional feeding groups ('FFG'; deposit feeders, filter feeders, suspension feeders, scavengers, carnivores, omnivores and grazers), and higher level taxonomic groups ('HLT'; e.g. higher level Order, Class or Phyla, with

the exception of polychaetes which were separated into two –groups based on mobility: sedentary and errant).

6.4.3 Assessment of recovery

Recovery level was determined by comparing the environmental parameters from the Cage and Reference stations using six different approaches, with varying levels of complexity (Table 6-1). Methods 1 and 2 involved plotting and visually comparing point in time estimates against background conditions. The mean values (with standard error) for all stations and times were overlaid with point-in-time 95 % confidence intervals for Reference stations as well as the natural range of conditions that was encountered over the course of the study, based on the 5^{th} and 95^{th} percentiles for all Reference station data.

Method 3 involved simple point-in-time statistical comparisons using nested models to assess differences between the Cage and Reference stations (factor: 'Treatment'), where Station was a random factor nested within Treatment. Equivalent models were constructed for univariate and multivariate analyses, the former using the 'aov()' function in R and the latter using the ANOSIM procedure.

Methods 4 and 5 used a test for 'parallelism' (forward and backward stepping) following the methods of Wiens and Parker (1995) and more recently Skalski et al. (2001) - also known as a level-by-time interaction. Parallelism assumes that after impact (in this case organic enrichment), control and impact profiles converge over time and eventually track (or parallel) each other as impacted sites begin to respond solely to the same regional climatic changes or oceanographic conditions as the reference sites. Hence, parallelism between mean profiles for (in this case) the impacted and Reference stations provides inferential evidence of recovery (Skalski 1995, Skalski et al. 2001). Population or community level differences between control and impacted sites are not considered in assessing recovery in this manner, only the relative patterns of the temporal trends (Skalski et al., 2001).

Parallelism analysis requires data to be analysed on a scale where natural differences between sites and temporal effects have an additive effect on population levels (Skalski et al., 2001). Where the strength of the response varies greatly, such as in population data, a transformation will be required to reduce any differences in amplitude; this also emphasises the trends such that tests for parallelism can be applied. Animal abundance (N) was the only

variable log-transformed; all other variables responded normally and/or the scale for change was constrained. A nested linear mixed effects model permitting random slope and intercept was constructed using the lmer() function in the nlme library in R (Zuur et al. 2009), where year ('Ye') was treated as a continuous variable, treatment ('Tr', Cages versus Refs) as a fixed factor, and station ('St') as a random factor. As such, the 'Ye× St (Tr)' interaction became the test for parallelism. The test was applied to a reduced time series, for example three consecutive surveys out of the 10 year dataset, starting with the first or last year sampled. If the interaction term was non-significant then the window was moved forward (if forward stepping), or back (if backward stepping) one year, and the test repeated (Table 6-1). The resulting P-value was overlaid on scatterplots of the environmental variables, using horizontal bars to display the windows over which the tests apply, and to assist with visualisation of the results.

A comparable multivariate model was also constructed using Permutational analysis of variance (PERMANOVA+ for PRIMER, Anderson 2008), this approach tested for recovery in square-root transformed macrofauna composition data (MCD), FFG, HLT count data, and also the collective influence of all the univariate environmental variables combined ('*All Vars*'). *All Vars* analysis was undertaken using Euclidean distances, with data first normalised ((x – SD)/SD) to account for differing scales and arbitrary origins (Clarke 2006). Differences between Cage and Reference stations through time were tested using a three-factor nested repeated measures design: factor 1 = Year ('Ye', 8 levels, fixed), factor 2 = Treatment ('Tr', 2 levels, fixed), factor 3 = Station(Treatment) ('St(Tr)', 4 levels, random). Significant terms were further investigated where required using *a posteriori* pairwise comparisons with 9999 permutations. Type I SS (sequential) were used, as some of the subsets were unbalanced. Further analysis of the components of variation for terms of interest was undertaken by calculating the distance among centroids in PERMANOVA and plotting the resulting matrix using principle coordinates (PCO, Anderson 2008).

Table 6-1: Definitions of the six different approaches used to evaluate recovery.

Method	Definition
1. Long-term background range:	The point in time that the mean value for the Cage stations first falls within the overall background range of conditions at the Reference stations and remains there for the remainder of the study.
2. Point-in-time background range:	The point in time that the standard error bars for the Cage stations first overlaps with the point-in-time percentiles for the Reference stations and remains there for two or more consecutive years.
3. Point-in-time ANOVA/ PERMANOVA	The first point at which the Cage stations are considered statistically comparable ($P<0.05$) to the Reference stations using nested ANOVA or PERMANOVA (Factors: Treatment, Station(Treatment)).
4. Parallelism- forward stepping:	The mid-point of the first time window for which a non-significant ($P>0.05$) interaction term is obtained, moving forward in time (from T0 to T8). Conducted for different length time windows.
5. Parallelism- backward stepping:	The mid-point of the last time window for which a non-significant ($P>0.05$) interaction term is obtained, moving backward in time (from T8 to T0). Conducted for different length time windows.
6. Biological and chemical remediation (from Brooks et al., 2003)	Chemical: "the reduction of accumulated organic matter with a concomitant decrease in free sediment sulphide and an increase in sediment redox potential under and adjacent to salmon farms to levels at which more than half the reference area taxa can recruit and survive" Biological: "the restructuring of the infaunal community to include those taxa whose individual abundance equals or exceeds 1% of the total abundance at a local reference station. Recruitment of rare species representing <1% of the reference abundance is not considered necessary for complete biological remediation."

6.5 Results

6.5.1 Spatial and temporal patterns in indicator variables

Prior to fallowing (i.e. T-2 to T0), the seabed beneath the cages was highly impacted (Figure 6-2). Organic matter content was markedly elevated (%OM 15 to 20%, Figure 6-2a) and the macrofaunal community was impoverished with few enrichment tolerant taxa remaining (predominantly *Capitella capitata*) (Figure 6-2c). The BQI was low (0.5 to 1.5), and the AMBI was relatively high (3.4 to 5.8), indicative of "bad benthic quality" and "poor ecological" quality statuses respectively (Figure 6-2d,e). Total abundance (N) was reduced with an average of 5

individuals at T-2 and 31 to 40 individuals at T0, compared with a range of 40 to 120 individuals at the Reference sites (Figure 6-2b). Average ES at T0 was 5.6 indicating highly enriched overall conditions (Keeley et al. 2012a). Seabed impacts were highly localised, with a marked reduction in %OM to near-background levels (4 to 7 %) within ~25 m of the cages (Figure 6-2a). Macrofauna composition improved markedly within the first 25 m, continuing to improve with increasing distance from the Cage stations. Nonetheless, an effect was still clearly evident (low S, H' and BQI) ≥ 100 m from the cages.

One year after fallowing, %OM at the fallowed Cage stations was approximately 20% lower (%OM = 12%) than that observed at T-2. Over the same time period, there was considerable improvement in the biological indicators: S increased from approximately 3 to 16 taxa per core, and the AMBI and BQI biotic indices indicated an improvement to an 'unbalanced' or 'meanly' polluted state and to poor benthic quality, respectively (Figure 6-2d,e). According to the main biological indicators (i.e. log(N), S, AMBI, BQI) recovery over the next two years (T2 and T3) was negligible, although %OM continued to decrease to around 10%. In the fourth year of recovery, most of the indicators (%OM, S, AMBI, M-AMBI) showed further substantial improvement at the fallowed Cage stations, achieving levels comparable to those found at the Reference stations. An exception was the BQI, which although improved (to BQI ≈ 8), was not yet comparable to background levels (BQI 9 to 10). At T5 a slight deterioration was evident, particularly in S and BQI. However, between surveys T6 and T8, all results (except BQI) suggested that conditions were similar to the Reference stations. BQI scores continued to indicate an impacted state; an average of ~7 at the Fallowed-Cage stations compared with 8 to 11 at the Reference stations (Figure 6-2e).

Unexpectedly, there was an apparent increase in enrichment at the Gradient stations (especially 25 m) midway through the study, mainly associated with the distribution of %OM. Initially the peak in %OM was at the Cage stations, but after 3 years (T3) this peak had shifted outwards to the 25 m station, where it remained for the following 4 years (Figure 6-2). An increase in %OM was also evident at the 50 m station at T5, but to a lesser degree. The biology appeared to follow a similar temporal and spatial response pattern; at T4 there was a peak in N at the 25m station and a general increase in N and S at the 75 m and 100 m stations. Notably, S also peaked temporarily at both the 100 m and Fallowed-Cage stations at T4. The biotic indices responded similarly with an initial increase in AMBI, decreased M-AMBI and BQI at ~50 m from the cages. AMBI and M-AMBI improved to levels comparable to the Reference

stations at T4, but once again BQI remained elevated, suggesting that macrofaunal composition was still impacted.

6.5.2 Patterns in macrofaunal composition

Multivariate analysis of the macrofaunal count data for all stations and times suggests a progressive convergence with time (T0 to T8, moving from right to left on Figure 6-3), as the fallowed Cage and near-cage (25 m) samples became increasingly similar to the Reference samples. Differences between years at the Reference stations were comparatively small. Recovery in the macrofauna composition at the Cage stations was greatest in the first year after fallowing (T0 - T1 R Statistic = 0.49, P<0.01, Figure 6-3), with S reaching approximately 50% of that observed at T8 (Figure 6-2b). The magnitude of recovery diminished in each subsequent year until T4, after which changes in community composition were relatively minor.

Considerable recovery was also evident at the 25 m station between T0 and T1 (Figure 6-3). However, the changes in the community observed at T3 and T4 did not follow the expected recovery trajectory, as the community became more dissimilar to the Reference stations than that observed at T1. Substantial recovery occurred between T4 and T5, with the 25 m stations becoming comparable to Reference stations (T5, 25 m – Reference, R Statistic = 0.43, P=0.13). Macrofaunal composition at the 75 m station was initially significantly different from both the Cage and Reference stations (i.e. at T-2, R Statistics > 0.91, P=0.1), but comparable to the 25 m station (R Statistic = 0.14, P = 0.8). There was substantial recovery at the 75 m station in the first year (T0 to T1), but minimal change thereafter (Figure 6-3), as the community was comparable to the Reference stations from T1 onwards (R Statistic < 0.6, P>0.1).

The difference between T0 and T1 at the fallowed Cage stations was mostly due to a shift from a community dominated by *Capitella capitata* (a first-order opportunist, Eco-Group V) to one dominated by Eco-Group IV (second-order opportunists: dorvilleid polychaetes, nematodes) and enrichment tolerant taxa (*Theora lubrica*, amphipods, and *Arthritica bifurca;* EcoGroup III, Figure 6-4). Abundances of *C. capitata* decreased to the point of not being a significant component of the assemblage at T2 and then disappeared altogether at T4. Nematodes decreased in abundance at T2, but increased at T3, before again declining

markedly and disappearing at T6. Second-order opportunists and enrichment tolerant taxa *(T. lubrica*, dorvilleid polychaetes, *Prionospio* sp. and amphipods) all continued to increase in abundance at T2 and T3. *T. lubrica* and *Prionospio* sp. remained dominant taxa through to the conclusion of the study (T8), whereas the importance of dorvilleid polychaetes diminished at T5 (Figure 6-4). Several Eco-Group II and III taxa, including polychaetes (belonging to the Families Glyceridae, Lumbrineridae, Hesionidae, Flabelligeridae and Trichobranchidae) and cumaceans, were important at T2. The numerical importance of most of these taxa was short lived, with the exception of lumbrinerid polychaetes, which were important contributors to the similarity of the assemblages throughout.

While the abundance of many early colonizers decreased at T3, cirratulid polychaetes, *Sphaerosyllis* sp., maldanid polychaetes and *Heteromastus filliformis* became important contributors for the first time (Figure 6-4). Cirratulids , *Sphaerosyllis* sp. and maldanids (an EcoGroup I taxa) continued to increase in abundance at T4, at which point paraonid polychaetes, *Tanaid* sp., sigalionid polychaetes and *Nucula gallinacea* (an EcoGroup I bivalve) became notable components of the assemblage for the first time in the recovery phase, with cirratulids and paraonids being dominant taxa for the remainder of the study. *Cossura consimilis*, brittle stars (Ophiuroidea) and terebellid polychaetes all featured prominently late in the recovery process (T5 and T6), and are taxa which are either considered sensitive, or indifferent to enrichment. *Myriochele* sp. (an EcoGroup III taxa) and isopods (Asselota) became prevalent for the first time at T8.

A relatively small shift in background or 'natural' conditions was observed in the macrofauna count data between T0 and T8 (Figure 6-3), principally due (in reducing order of importance) to reduced abundances of: ophiuroids, *Nucula nitidula*, *T. lubrica*, Spionidae, *Cadulus teliger, Echinocardium cordatum* and *Neilo australis*, and increased abundances of paraonids and *C. consimilis*.

Multivariate analysis of FFG data showed substantial recovery in the first year, followed by a high degree of temporal (inter-annual) variability, due to fluctuations in the relative abundances of scavengers, omnivores and carnivores (Figure 6-5a). The temporal changes in HLT groups were largest in the first 2 years, as the community shifted from being highly dominated by sedentary polychaetes and nematodes, to one with a more balanced assemblage of errant polychaetes, crustaceans, bivalves and amphipods (Figure 6-5b). At the

conclusion of the study (T8), the HLT composition of macrofauna at the fallowed Cage and Reference stations was very similar. Although the composition of the Reference station taxa also changed through time, the direction was different to that of the main recovery pathway, and was mainly associated with a reduction in numbers of echinoderms, gastropods, scaphopods and bivalves between T0 and T8 (Figure 6-5b). At a taxa level, the differences at T8 were mostly due to higher abundances of *Myriochele* sp. *Tanaid* sp., *T. lubrica*, *Prionospio aucklandica* and *H. filiformis*, and lower numbers of maldanids, *Ennucula strangei*, flabelligerid and Prialula polychaetes at the fallowed Cage stations (Figure 6-4). Of the 30 dominant taxa identified by the SIMPER analysis (Figure 6-4), only 6 were not common between the Cages and reference stations at T8, interestingly, 2 of which were first order opportunists that were present only at the References stations.

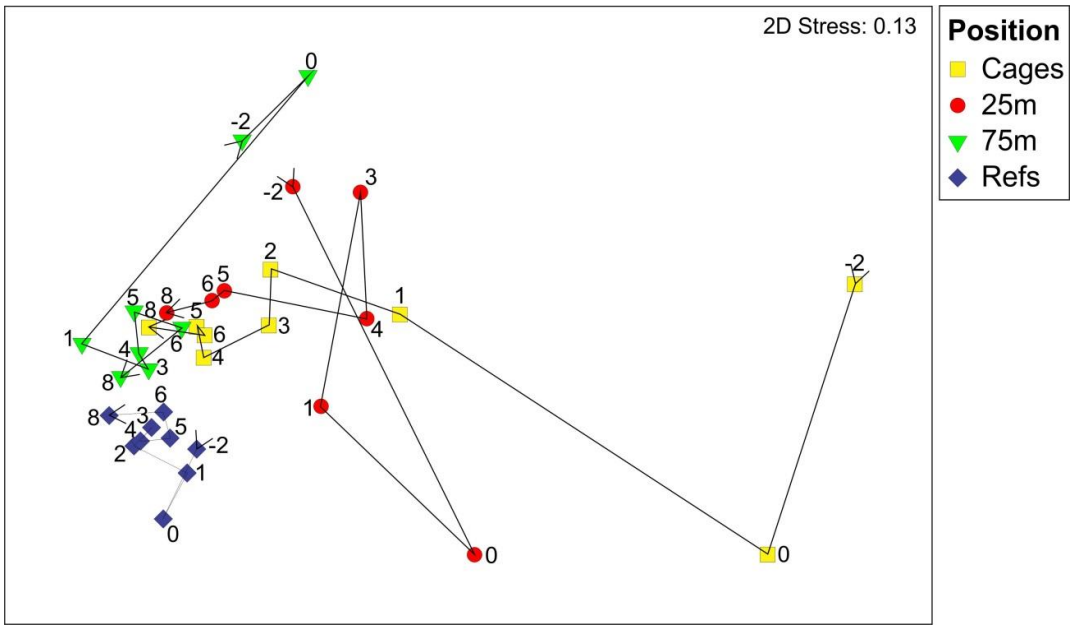


Figure 6-3: MDS ordination of FOR long-term time-series data (T-1 to T8, T0 = year of fallowing) for 'Cage', 25 m, 75 m and 'Reference' stations, based on Bray-Curtis similarities of station-averaged, square-root transformed macrofauna count data.

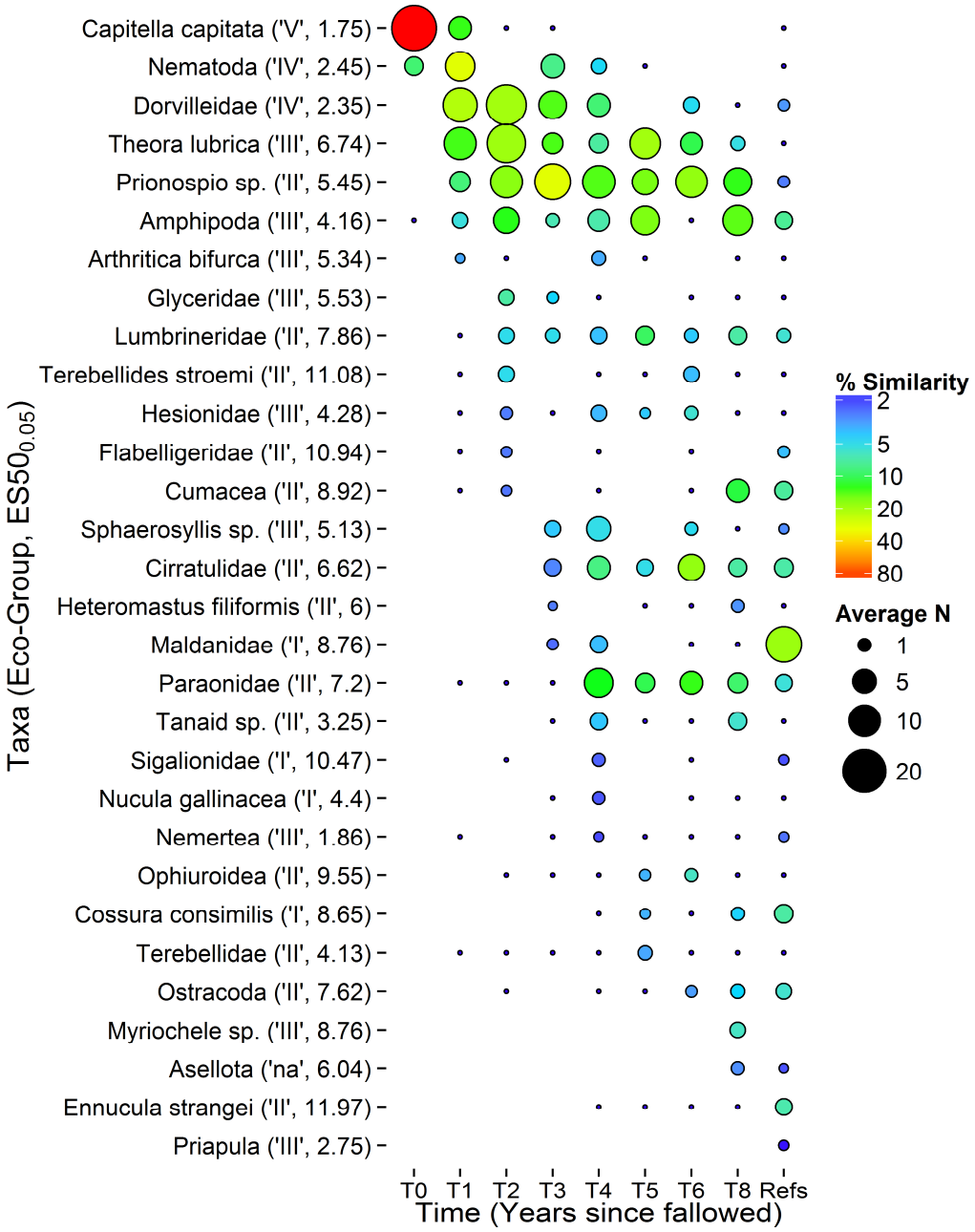


Figure 6-4: MDS ordination of FOR long-term time-series data (T-1 to T8, T0 = year of fallowing) for 'Cage', 25 m, 75 m and 'Reference' stations, based on Bray-Curtis similarities of station-averaged, square-root transformed macrofauna count data.

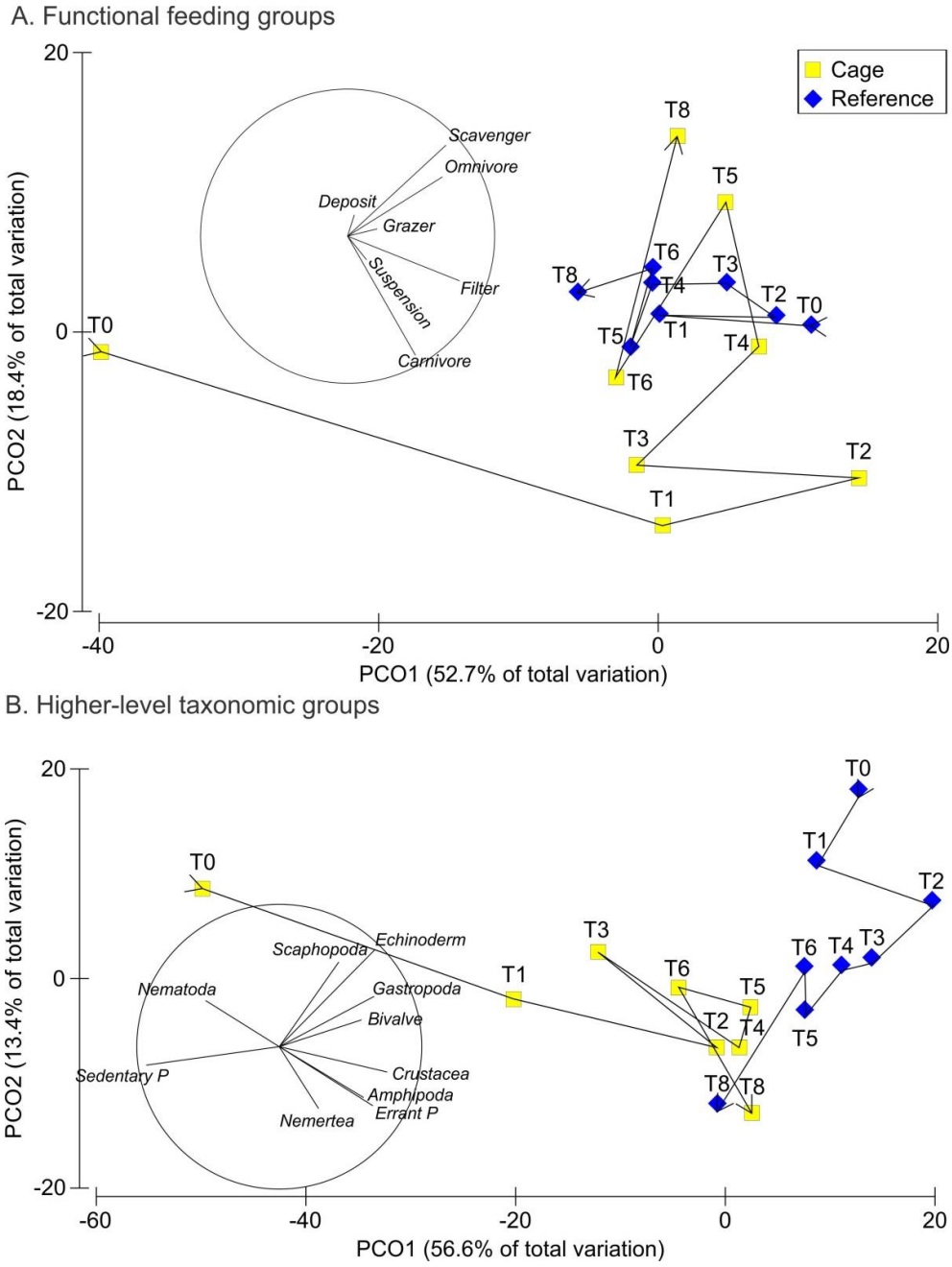


Figure 6-5: PCO ordination of Distance among Centroids for Cage and Reference stations from T0 to T8, based on Bray-Curtis similarities (+d) of square-root transformed macrofauna count data grouped according to a) functional feeding groups, and b) higher-level taxonomic groups. Overlaid with Pearson correlation vectors to indicate main drivers of differences in 2-Dimensional space..

6.5.3 Assessment against recovery criteria

The variables which most consistently depicted recovery were S, BQI, ES and *All Vars* (all variables assessed using multivariate analyses, Table 6-2). Recovery according to these variables was generally shown to have been achieved after 4 years; however, the range greater for S and ES (2 to 6 years) and for *All Vars* (2 to 4.5 years). AMBI and MCD also indicated time lengths between 4 and 5.5 years, but also that recovery had not been achieved in some instances (i.e. >5.5 years). The most commonly occurring time estimate (indicated by the mode) across all variables was 4 to 5.5 years. Note that because the midpoint of the time window was used as the 'recovery point' for the parallelism method, the maximum recovery timeline was effectively 5.5, 6 and 6.5 years for the 3, 4 and 5 year windows, respectively.

In terms of method-specific differences, the background range methods and the point in time ANOVA/ PERMANOVA tests were reasonably consistent in indicating that recovery had occurred after 4 years (Table 6-2). The most common recovery time estimates for the forward stepping parallelism method was 2, 4.5 and 5.5 years for the 3-, 4- and 5-year windows respectively. It was also apparent that the 2 year estimate from the 3-year time windows usually indicated an early, temporary stage of parallelism (Figures 6 - 8). Although the temporal responses were similar over this period, the indicator levels remained substantially different, and the subsequent time window (T2 – T5) indicated further significant change at the fallowed Cage stations. When the slightly longer 4-year window was used to test for parallelism, the recovery that had been apparent during T1-T3 using the 3-year window was no longer evident (Table 6-2, Figures 6 - 8).

The most common time estimates using the backward stepping method was >5.5 years, suggesting recovery had not occurred during the study period. However, periods of parallelism were evident earlier in the dataset, highlighting a possible issue with the backward stepping approach. In the case of the AMBI and BQI indices, this was due to a small divergence and reduced sample variability, increasing statistical power. This divergence was mainly due to improving conditions at the control stations (decreasing AMBI and increasing BQI), while the conditions at the fallowed Cage stations remained relatively static (Figures 7b and 8a). Similarly, although changes were still sometimes evident in the last time window for %OM, the significant difference was due to levels being lower at the Cage stations than at the Reference stations, consistent with a less enriched state at the farm site.

In several instances, the main term (either 'Tr', in the case of the point-in-time analyses, or the 'Ye×Tr' interaction for parallelism) was not significant early on, but the term involving Station nested within Treatment (i.e. 'St(Tr)', or 'Ye×St(Tr)') was highly significant (e.g. Appendix Table 6-A and 6-C). This was usually due to a large amount of variation in the factor Station(Treatment) masking differences in the main term. Further analysis of the components of variation for that term showed that the Cage Stations still changed substantially more than the reference Stations, but that the rate at which the Cage stations were changing was often different (e.g. Appendix Figure 6-A to 6-C). Unfortunately, recovery was greater at one station and as a consequence the main interaction term was not significant. In these instances discretion was used to select the point most indicative of recovery.

Total abundance (log transformed) generally indicated recovery early on in the study (mode = 2 years) suggesting that the fallowed Cage stations were comparable to the Reference stations at T0, despite obvious differences in many other variables. FFG also appears to suggest a relatively rapid recovery (only 1 year), but was inconsistent with later assessments suggesting recovery was not complete at the conclusion of the study.

In terms of biological remediation (Brooks et al., 2003, Method 6), a total of 19 dominant Reference station taxa, defined as those whose individual abundance equals or exceeds 1% of the total abundance were identified: amphipods, polychaete worms (*Cossura consimilis*, *Prionospio* sp., *Sphaerosyllis* sp., dorvilleids, cirratulids, hesionids, lumbrinerids, maldanids, paraonids, sigalionids,), nematodes, cumaceans, ophiuroids, ostracods, priapulid worms and small bivalves (*Theora lubrica*, *Nucula gallinacean* and *Ennucula strangei*). At T-2 and T0, only 2.6% and 4.4% of these taxa (respectively) were present at the Cage stations. Substantial recruitment occurred at T1 and T2 (26% and 41%, respectively) and then again at T4, at which point the level of colonization peaked, with 69% of the dominant Reference station taxa being present. This declined back to 48% at T5 and remained around 50% (±5%) through until the conclusion of the study. At T1, an average of 8.1 reference station taxa (i.e. found at the reference stations over the course of the study) were present at the Cage stations, which corresponded to 54 % of the reference station S at T1.

Table 6-2: Summary of recovery estimates for selected indicator variables and the multivariate analyses based on five different methods. *'Ye×Tr' non-significant at an earlier time, but 'Ye×St(Tr)' remained significant due to large between-cage Station differences (Appendix Tables 6-A to 6-C). Temporal difference at fallowed-Cage stations still considerably larger than for Reference stations until specified time (see Appendix).

	Univariate tests						Multivariate tests				
Method	**%OM**	**log(N)**	**S**	**AMBI**	**BQI**	**ES**	***All Vars***	**MCD**	**FFG**	**HLTG**	**MODE:**
1. Background range:	6-8	0	1	4	4	4	-	-	-	-	4
2. Point-in-time background:	6-8	2	4	>8	4	4	-	-	-	-	4
3. Point-in-time ANOVA/ PERMANOVA	4	0	4	5	4	4	4*	4*	1	4*	4
4. Parallelism - forward stepping:											
3-year windows:	2	3	2	5	2	2	3*	5*	1	1	2
4-year windows:	4.5	1.5	3.5	>6	>6	6	4.5*	4.5*	1	4.5*	4.5
5-year windows:	>5.5	2	4	5.5	5.5	>5.5	4*	>5.5	4*	>5.5	5.5
5. Parallelism - backward stepping:											
3-year windows:	>6.5	2	4	>6.5	>6.5	5	4*	5*	>6.5	5*	>6.5
4-year windows:	4.5	1.5	>6	>6	>6	6	3*	4.5*	>6	4.5*	>6
5-year windows:	>5.5	2	4	5.5	5.5	>5.5	2*	>5.5	>5.5	>5.5	>5.5
MODE:	4.5	2	4	5-6	4	4	4	4-5.5	1	4.5->5.5	

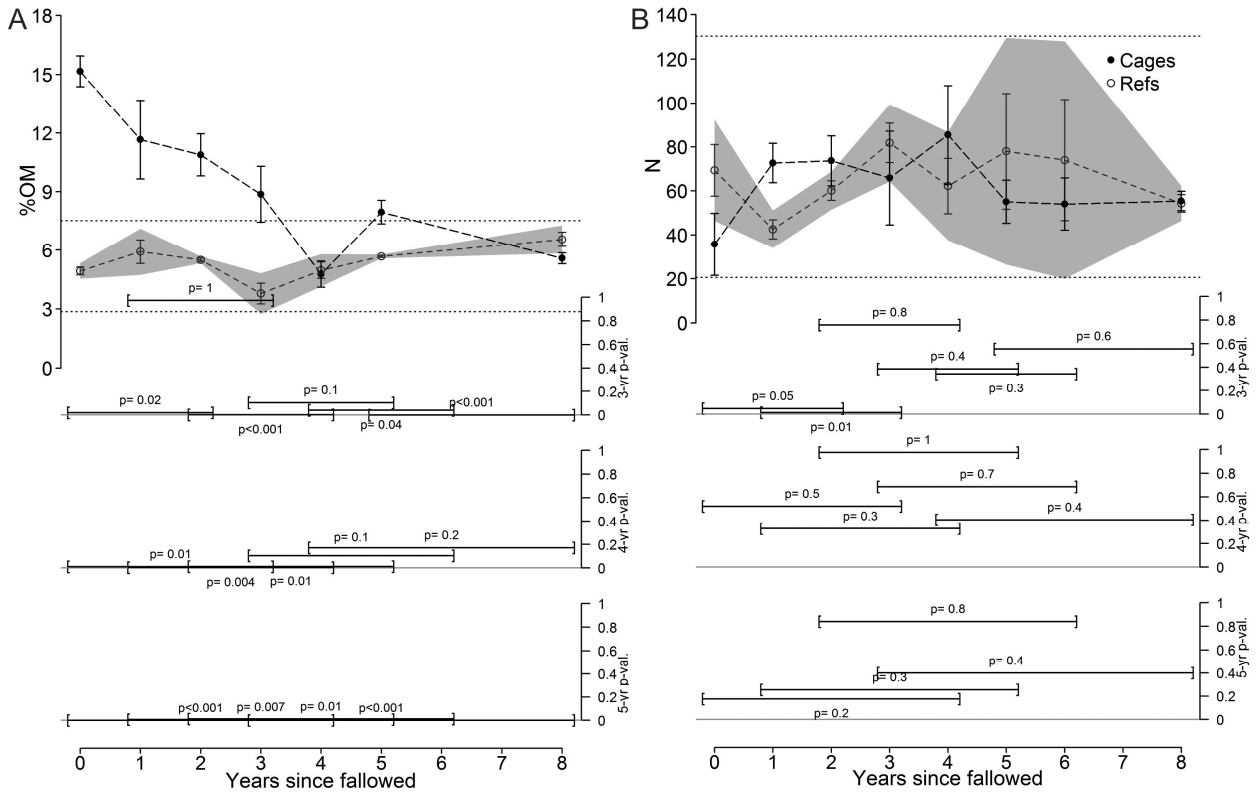


Figure 6-6: Comparison of temporal profiles of Cage and Reference stations for average: a) %OM and b) N (note, analyses conducted on log transformed data), with corresponding p-values indicating results of test for parallelism (i.e. Year×Treatment term) for 3, 4 and 5 year time windows (indicated by horizontal bars on bottom three y-axes). Y-axes correspond to specified p-value. Vertical error bars on main plots represent SE. Grey shaded area represents point-in-time 95% CI for Ref stations, and horizontal dashed lines indicate the range of background conditions over the entire study (5th and 95th percentiles for Ref Stations).

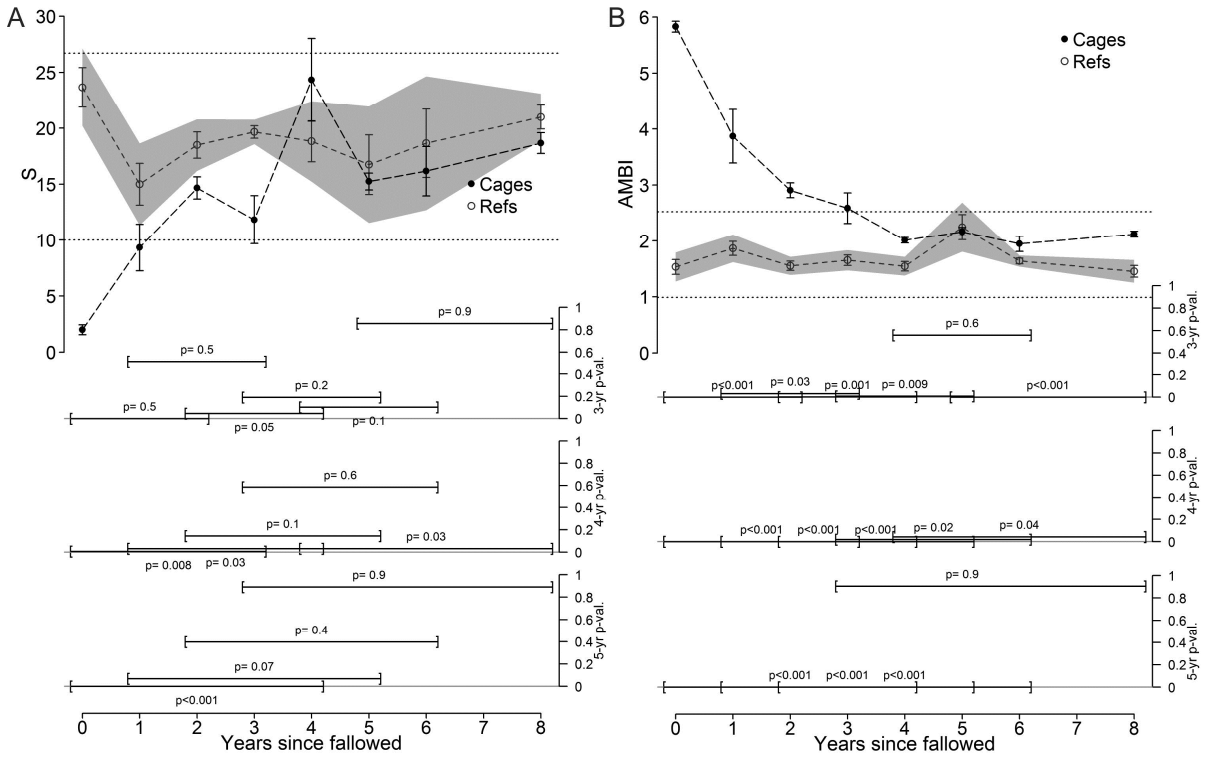


Figure 6-7: As in Figure 6-6, but for: A) S and B) AMBI.

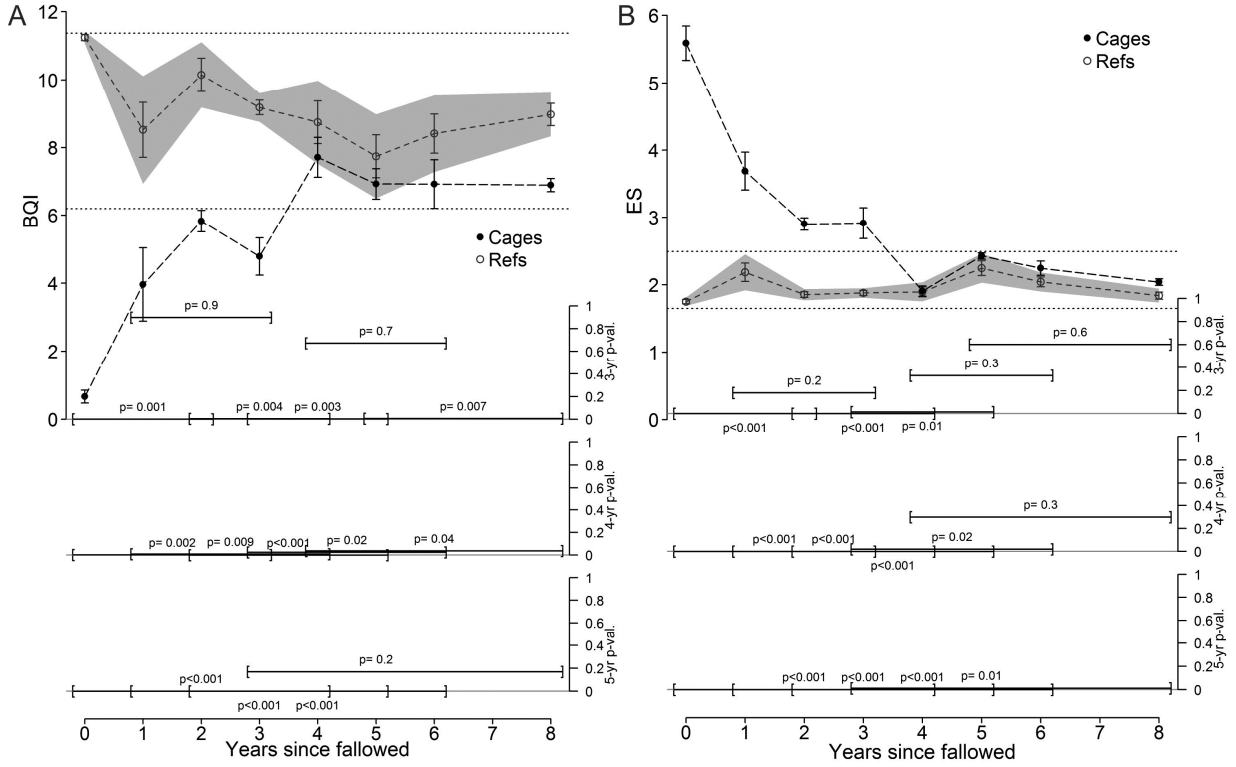


Figure 6-8: As in Figure 6-6, but for: A) BQI and B) ES.

6.6 Discussion

6.6.1 Recovery timeframes

The duration of this study (8 years) allowed for an evaluation of longer-term recovery processes and interactions that are not possible with shorter-term assessments where recovery has not been realised (Karakassis et al. 1999, Lin & Bailey-Brock 2008, Macleod et al. 2008, Villnas et al. 2011). Substantial improvement in seabed health beneath the farm site was observed in the first 2 years, followed by more gradual and variable-dependant improvements over the following 2 to 3 years. A weight-of-evidence assessment suggested that 'recovery' had ostensibly been achieved after 4 to 5.5 years. At this point sediment conditions were, in many respects, comparable to the Reference stations however, significant differences were still evident in some environmental indicators. The fact that recovery occurred in years rather than decades is significant and reinforces previous assertions that salmon farming is unlikely to have long-term adverse benthic impacts (e.g. Lu & Wu 1998).

Chemical remediation, as defined by Brooks et al. (2003), requires significant improvements in %OM and sediment chemistry (sulphides and redox) such that more than half the reference taxa can recruit and survive, and this consistently occurs earlier in the process than biological remediation. Although no chemical data was available in our study, %OM had clearly declined and the underpinning biological requirement was achieved after one year. This is a relatively long recovery period compared with some previous studies (Ritz et al. 1989, Brooks et al. 2003), which reported similar levels of remediation within a few weeks to 6 months, but was much shorter than the 5.4 years estimated for a biologically a-typical site where the macrofauna was dominated by bivalves (Brooks et al. 2004). Bivalves are typically suspension or deposit feeders and generally considered sensitive to enrichment (Pearson & Rosenberg 1978, Borja et al. 2000). However, it is important to note that this "a-typical" community also lacked opportunistic polychaetes, which are critical to the recovery process (Macleod et al. 2007).

One definition of biological remediation requires the complete re-establishment of the dominant taxa (>1% by abundance) at reference sites (Brooks et al. 2004). In the present study only 68% of the dominant Reference taxa had re-established after four years, and the level subsequently reduced with only ~50% re-establishment at the end of the study. Hence,

alternate definitions of recovery proposed by Brooks et al. (2003, 2004) can be applied at differing timescales and comparisons with the current data clearly shows that estimates of the timeframe for recovery not only vary markedly based on these definitions, but can also differ according to the local ecology.

6.6.2 Indicators of recovery

In terms of notable responses of individual taxa, the approximate point of 'recovery' (i.e. ~5 years) identified here coincided with large reductions in the abundance of nematodes and dorvilleid polychaetes. These enrichment tolerant taxa are prevalent under moderate-to-high levels of enrichment (ES 3 to 5, Keeley et al. 2012b) and therefore their substantive decline may be a useful indicator of biological remediation. At the same time, several other taxa became established as important components of the macrofauna (ophiuriods, the polychaete *Cossura consimilus*, members of the polychaete family Terebellidae, ostracods and the bivalve *Ennucula strangei*). Four of these are listed on the AMBI database (http://ambi.azti.es/) as EG I or II taxa and hence appear to be good "universal" indicators of unimpacted conditions (Borja et al., 2000) and recovery. There were also other taxa that were central components of the macrofauna in the initial phases, but then remained dominant throughout the recovery process and hence would not be useful indicators of recovery stage (e.g. the bivalve mollusc *Theora lubrica*, the polychaete *Prionospio* sp. and members of the polychaete family Lumbrineridae).

Unsurprisingly, the analyses highlighted the important role of *Capitella capitata* during moderate to severe enrichment (ES4 to 6) in the early stages of recovery (i.e. the first two years). However, it was interesting that despite being the dominant species in these early stages, there was not a strong peak in total abundance (N) as has been so frequently reported in association with benthic impacts (Pearson & Rosenberg 1978, Rosenberg et al. 2004, Hale & Heltshe 2008). Instead, N at the fallowed Cage stations remained statistically similar to the Reference stations throughout the study. Distinct differences in the proliferation of opportunists during recovery at site level have been observed elsewhere, but the reasons for this remain unclear (Brooks et al., 2004). In the current study this is possibly an artefact of sampling timing and frequency, which was annual, whereas the peak (PO) can occur over the first few months of recovery (e.g. Pereira et al., 2004). Nevertheless, the 'peak of

opportunists', and accordingly N, appears to be an unreliable indicator of ecological succession during recovery.

Consequently, although ecological differences are critical to defining change in response to organic enrichment it is risky to depend upon a few specific indicator species or more simplistic measures of ecological condition, particularly in the later stages of recovery. Beyond 3 years, impacts were less obvious and were mainly evident as compositional differences in the macrofauna and as a result, variables such as N and S tended to suggest recovery earlier on. The variables that were most consistent in their estimate of recovery tended to the more complex biotic indices, which take into account some aspect of the species identity or functional role (BQI and AMBI) and ES (which integrates across all variables - biotic and abiotic, Keeley et al., 2012a). The multivariate approach, which also integrated all variables, was similarly robust. This reinforces previous findings that Eco-Group based indices, the BQI and ES are most useful for discerning enrichment effects associated with finfish aquaculture (Borja et al., 2009; Keeley et al., 2012a) and medium-term recovery states (Borja et al., 2006). The simplistic individual variables tended to be more susceptible to variable-specific and non-intuitive responses.

The detailed assessment of recovery here is based primarily on the state of the seabed at the fallowed Cage stations and it is conceivable that some of the variability observed may be an artefact of sampling and spatial variability rather than temporal changes *per se*. Field observations during the last survey indicated some residual small-scale patchiness at the Cage stations, with small pockets of blackened (anoxic) sediments amongst natural sediments. Although this may have contributed to individual sample variability, the triplicate samples collected on most occasions should have accounted for the small-scale patchiness, and provided a reasonable estimate of overall condition. However, the spatial analyses did suggest a larger-scale patchiness and 'shifting' of the enrichment peak that may explain some of the inter-annual variability. The peak in %OM shifted to outside of the historical Cage footprint (25 to 50 m away) after 3 to 4 years, where there was a corresponding biological response, resulting in a higher overall enrichment stage (ES). Interestingly, at the same time the total number of taxa increased at both the Cage station, and further away at the 100 m position. This observation is somewhat perplexing, as there was no known new source of external organic matter at the Gradient stations, and considerable care was taken to accurately relocate sampling stations. One possible explanation is that the predominant current flow in

the direction of the Gradient stations gradually transported organic material in that direction, the result being a slowly migrating enrichment peak. Simultaneously, the elevated densities of opportunists, especially at the 25 m station, may have themselves contributed to the apparent increase in organic content of the sediment samples. Regardless of the cause, these reasonably large-scale spatial and temporal patterns in the benthos could be responsible for both the variability observed in some indicators at the Cage stations (e.g. functional feeding groups), and potentially some of the significant Ye×Tr interactions that occurred later in the study.

6.6.3 End-points and methods for assessing recovery

Compositional disparity between fallowed Cage and Reference station communities can be interpreted as a failure of the simplest criteria for recovery. However, this assumes both steady state and spatial equilibrium, and the related successional theory that there is only one 'climax' state, to which impacted communities will return - which is generally considered to be an overly simplistic view (Beisner et al., 2003; McCook, 1994; Parker and Wiens, 2005; Young et al., 2001). In this study, failure to converge on a similar endpoint was most evident in the AMBI, BQI and the multivariate analyses of the macrofauna assemblage. Such compositional differences in the latter stages of recovery are often attributable to the absence of late successional 'equilibrium' (or climax) species that tend to have slower re-colonisation rates (Whitlach et al., 2001), or may be excluded by early colonisers (Connel and Slatyer, 1977). However, in this case the differences observed at the conclusion of the study were mainly due to differences in the relative abundances of similar taxa, most of which had comparable ecological functions. Furthermore, the assumption of steady state equilibrium was clearly not valid in this instance as there was a shift in reference conditions over the course of the study, which is evident in both the macrofauna count data and the high-level taxonomic grouping analysis. Therefore, the concepts of a dynamic equilibrium (Skalski et al., 2001) and alternative stable states (Beisner et al., 2003) appear to be applicable in this particular assessment of 'recovery'.

The point at which communities have a broadly equivalent faunal composition with similar functional roles (e.g. bioturbation, feeding and reproduction strategies) has been proposed as a useful reference point for recovery (Macleod et al., 2008). The critical aspect in

this context being that the biological and ecological characteristics achieve a state from which the system has "the capacity" to fully recover (Macleod et al., 2008). Multivariate analysis and tests for parallelism of the functional feeding groups in this study revealed some on-going differences between Cage and Reference stations. Closer analysis of the data revealed that although the functional composition of the macrofauna was reasonably similar to the reference communities after two years, there were significant interactions observed in the parallelism tests due to alternations in the relative dominance of two or three of the main feeding groups. Such compositional 'instability' can occur post-disturbance if the habitat is modified such that it favours recolonisation by species other than those which previously existed (Connel and Slatyer, 1977). Thus ecosystem function appears to have been restored relatively quickly, but on-going compositional instability suggested a stable state had not yet been achieved (Connel and Slatyer, 1977). In some situations this may be indicative of impacted sediments (Karakassis et al., 1999; Mendez and Linke-Gamenick, 2001).

Parallelism is one approach that can be used to assess recovery over the longer-term, and is particularly appropriate where communities may be subject to natural changes. The basic premise of the test for parallelism is that impact and control sites will begin to track, or "parallel", each other when the influence of the impact is no longer important and they are both responding, solely to wider environmental stimuli (Parker and Wiens, 2005; Skalski et al., 2001). Most of the individual indicator variables met this criterion over the 4 to 5.5 year range. However, several of the biotic indices failed the test (i.e. the null hypothesis was rejected) when the last survey was included in the assessment window - particularly when the backward stepping method was applied. In the context of the greater recovery trajectory, this divergence in the last time window was usually relatively small, due to deterioration at the Reference stations (as opposed to on-going improvements at the Cage Stations), and statistical significance was aided by the fact that variability among the Cage samples was generally reduced in the final year. Hence, it was not always consistent with incomplete recovery. This highlights a weakness that exists in both the forward and backward stepping approaches. When the backward stepping approach is applied, parallelism is assumed to have never occurred, when in fact a period of similar responses may have occurred earlier in the study. Conversely, with the forward stepping procedure parallelism can be concluded prematurely.

Window size is an important consideration when applying the parallelism test as it represents a compromise between power (longer time windows have higher degrees of

freedom for the error term) and temporal resolution (longer windows being less sensitive to localized deviations from parallelism) (Skalski et al., 2001). Indeed, using longer (5 year) time windows here increased the ability to reject the null hypothesis at the conclusion of the study (i.e. including years 6 and 8), i.e. indicating that parallelism had not been achieved. Similarly, the shortest 3-year window identified parallelism at an early stage for most variables, between years 1 and 3, but this was rejected as evidence of recovery because subsequent windows did not demonstrate parallelism and the full range of temporal plots indicated that such a finding was premature. Thus, longer time windows are less prone to falsely identifying parallelism. However, a disadvantage to using longer time windows is that it is more difficult to nominate a single year as being the point of 'recovery' and requires monitoring to be conducted for a longer period. The recommended solution is to combine parallelism and visual assessment methods by identify all stages of parallelism and then selecting the first period during which all points within the window remained within the range of natural background variability. This approach is most reliable when applied to more complex biotic indices and metrics that unify multiple variables.

The above highlights the numerous unresolved challenges to reliably determining recovery. A fundamental issue is the lack of clarity and consensus around what constitutes 'recovery', and hence this subject requires further attention by scientists and environment managers. Even if a definition or end-point for recovery is agreed upon, determining whether recovery has been achieved is complex and requires expert judgement. Therefore, for the foreseeable future, multiple indicators of seabed recovery should be monitored and a weight-of-evidence approach applied.

6.7 Acknowledgements

This research was made possible through internal investment funding (IIF) from the Cawthron Institute (Nelson, New Zealand), additional support from Institute of Marine and Antarctic Sciences (IMAS), University of Tasmania, and from the New Zealand King Salmon Company Ltd., who kindly made available much of the source data. As the study was conducted over an eight year timeframe, several people have been involved in the collection of the data and the maintenance of the program over the years; including Dr Steven Brown, Murray Clark, Reid Forrest and Dr Robyn Dunmore. Taxonomic analysis was undertaken by Rod Asher, Bill Nikkel and Fiona Gower, also of the Cawthron Institute. Valuable statistical advice was provided by Dr Elena Ieno (Highland Statistics Ltd, Scotland) and Associate Professor Marti Anderson (University of Auckland, New Zealand).

6.8 Appendix

Appendix Table 6-A: Main results of point-in-time ANOVA and PERMANOVA tests between Cage and Reference stations for univariate and multivariate variables (respectively). 'MCD' = fourth-root transformed macrofauna count data. 'FFG' and 'HLTG' are from square-root transformed counts based on 'functional feeding groups', and 'higher level taxonomic groups' (respectively). Significance for factors Treatment (Cage, Reference) and Station(Treatment) at each point in time, indicated by '·' = P<0.1, '*' = P<0.05, '**' = P<0.01, '***' = P< 0.001.

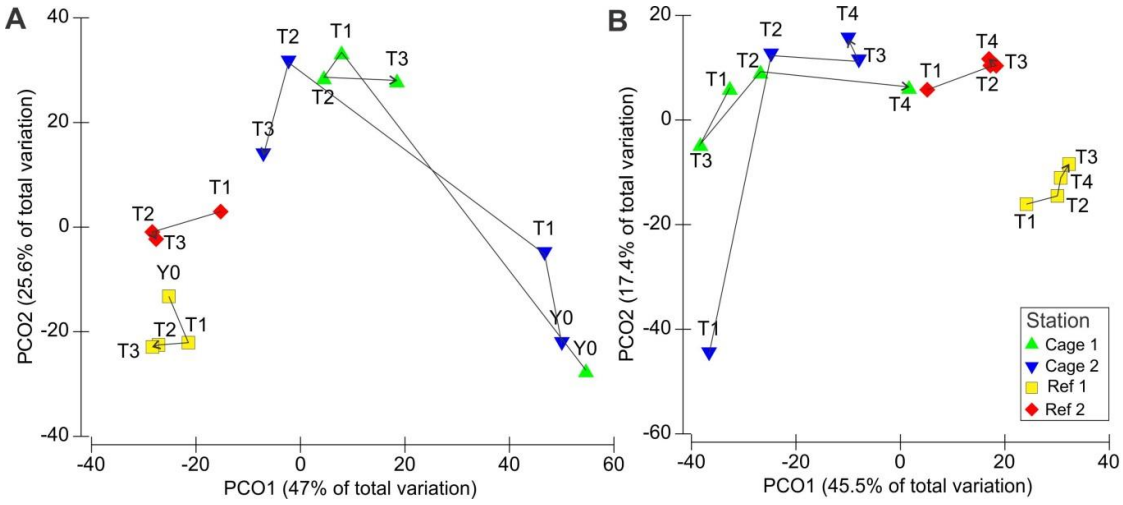
		ANOVA							PERMANOVA			
Year	**Factor**	**%OM**	**N**	**S**	**AMBI**	**M-AMBI**	**BQI**	**ES**	***All Vars***	**MCD**	**FFG**	**HLTG**
T0	Tr	***		***	***	***	***	***	*	*	*	*
	St(Tr)											
T1	Tr	***	*	*	***	***	***	***				
	St(Tr)	***		**	***	***	***	**	***	***	**	*
T2	Tr	***		*	***	***	***	***	*	*		*
	St(Tr)	**						*		*		*
T3	Tr	***		**	**	***	***	***	.	.		
	St(Tr)	***			*			*		**		.
T4	Tr				***							
	St(Tr)	*		.	**		*		.	*	*	*
T5	Tr	**	*									
	St(Tr)		**		*		.		**	*	*	.
T6	Tr				**							
	St(Tr)				**							
T8	Tr	***			***	*	***			*	*	
	St(Tr)	**			**		**	*	.			.

Appendix Table 6-B: Main results of tests for parallelism between Cage and Reference stations using random slope and intercept nested linear mixed effects models for 3, 4 and 5 year time windows. Significance of Year×Treatment interaction term indicated by: P < 0.1 = '·', P <0.05 = '*', P <0.01 = '**' and P < 0.001 = '***'.

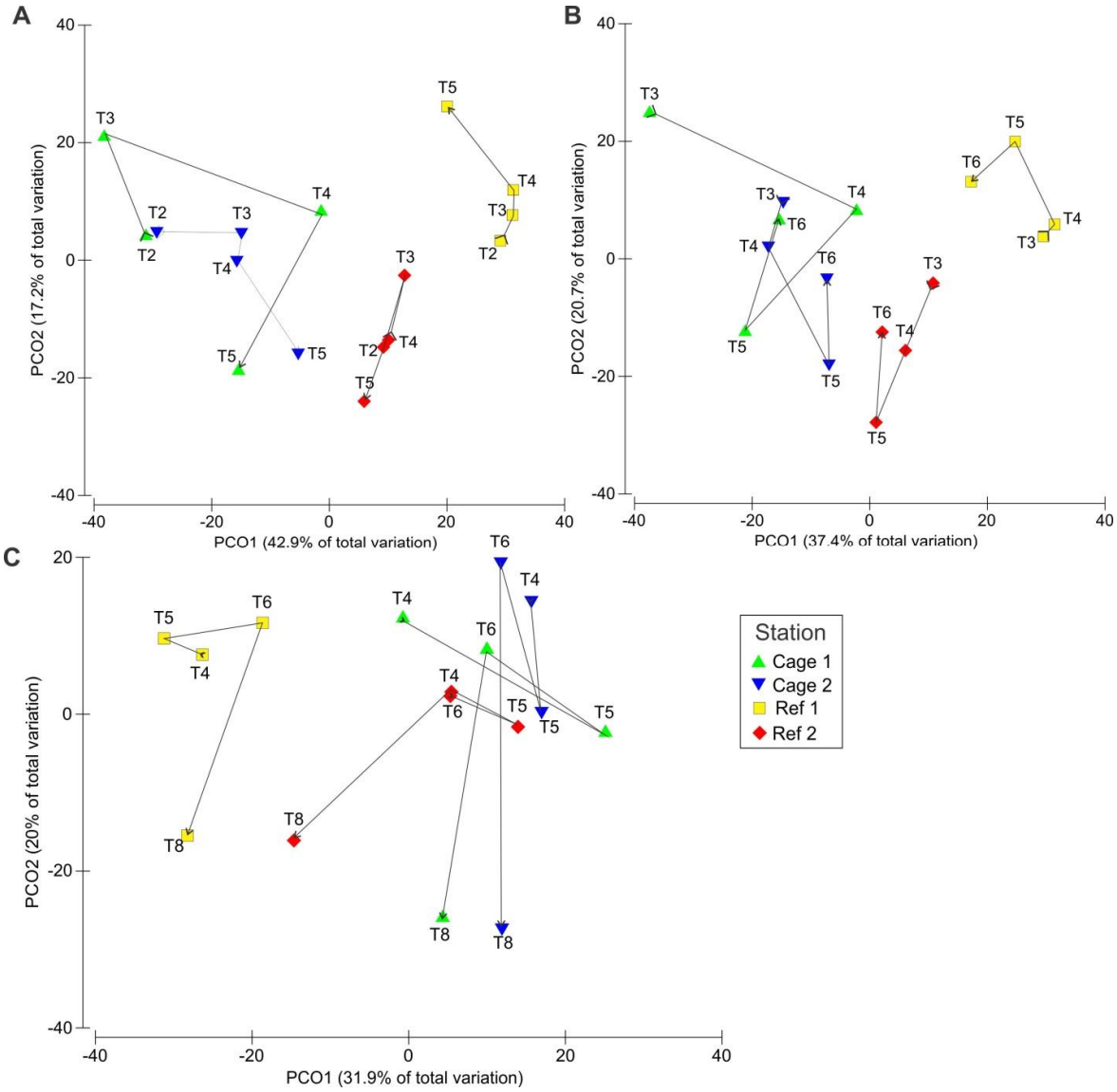
	Time window	%OM	log(N)	S	H'	AMBI	M-AMBI	BQI	ES
3-yr	T0-T2	*	*	***	***	***	***	**	***
	T1-T3		*		.	*			
	T2-T4	***		*	***	**	**	**	***
	T3-T5				*	**	*	**	*
	T4-T6	***							
	T5-T8	***			*	***		**	
4-yr	T0-T3	*		**	***	***	***	**	***
	T1-T4	**		*	***	***	***	**	***
	T2-T5	*			**	***	**	***	***
	T3-T6					*		*	*
	T4-T8			*	.	*	*	*	
5-yr	T0-T4	***		***	***	***	***	***	***
	T1-T5	**		.	***	***	***	***	***
	T2-T6	*			**	***	*	***	***
	T3-T8	***							*
All years		*		*	**	**	**	*	**

Appendix Table 6-C: Main results of tests for parallelism between Cage and Reference stations using multivariate data analysed using 3-factor nested models in PERMANOVA for 3, 4 and 5 year time windows. 'All' = all indicator variables (%OM, N, S, H', AMBI, M-AMBI and BQI), normalised and combined using Euclidian distance. 'MCD' = fourth-root transformed macrofauna count data, 'FFG' and 'HLTG' are square-root transformed counts based on 'functional feeding groups', and 'higher level taxonomic groups' (respectively). Significance of interaction terms indicated by: P < 0.1 = '·', P <0.05 = '*', P <0.01 = '**' and P < 0.001 = '***'.

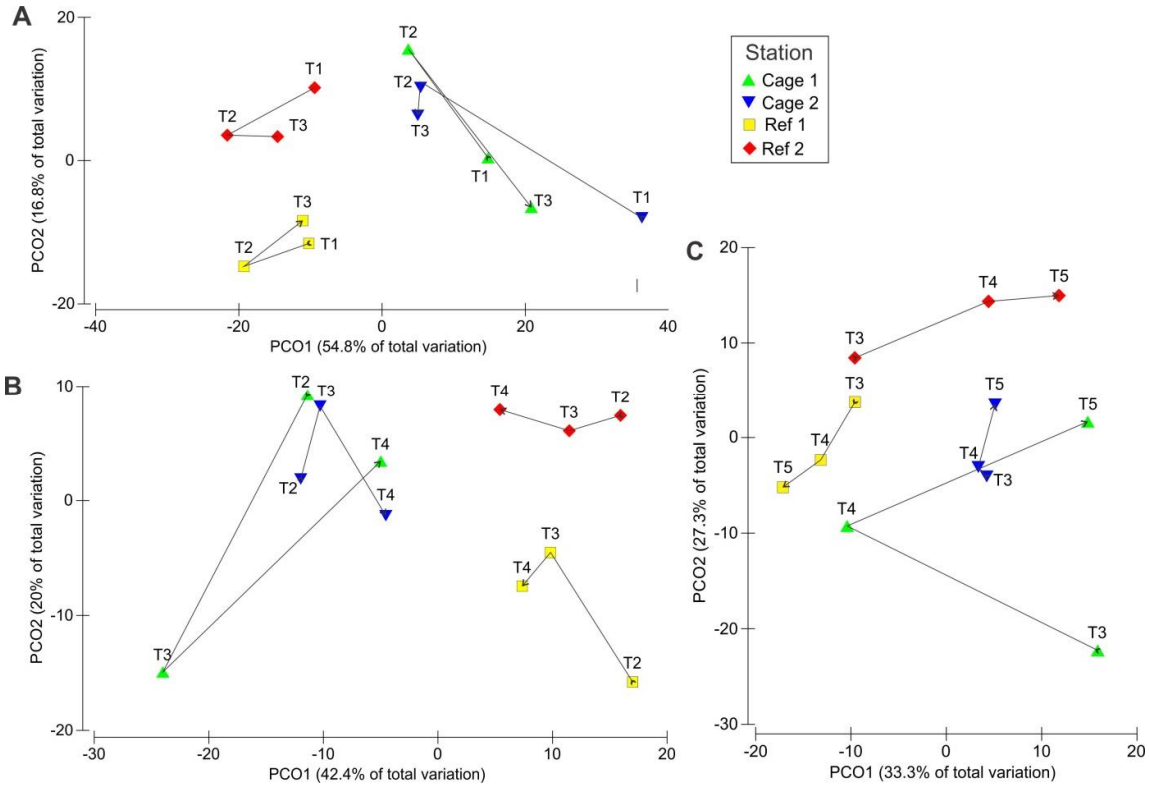
	Test period	Term	All	MCD	FFG	HLTG
3-Year windows	**T0-T2**	Ye×Tr				.
		Ye×St(Tr)	***	***	*	.
	T1-T3	Ye×Tr				
		Ye×St(Tr)	**	***	*	***
	T2-T4	Ye×Tr				
		Ye×St(Tr)	*	***	.	**
	T3-T5	Ye×Tr			.	
		Ye×St(Tr)	**	***	.	**
	T4-T6	Ye×Tr				
		Ye×St(Tr)				
	T5-T8	Ye×Tr			*	
		Ye×St(Tr)		.		
4-Year windows	**T0-T3**	Ye×Tr	.		.	.
		Ye×St(Tr)	***	***	.	*
	T1-T4	Ye×Tr				
		Ye×St(Tr)	***	***	***	***
	T2-T5	Ye×Tr			.	
		Ye×St(Tr)	**	***	.	**
	T3-T6	Ye×Tr			.	
		Ye×St(Tr)		**		
	T4-T8	Ye×Tr		.	*	
		Ye×St(Tr)		*		
5-Year windows	**T0-T4**	Ye×Tr	*	*	*	*
		Ye×St(Tr)	***	***	*	**
	T1-T5	Ye×Tr				
		Ye×St(Tr)	***	***	***	***
	T2-T6	Ye×Tr		.	.	
		Ye×St(Tr)		***		*
	T3-T8	Ye×Tr		*	**	.
		Ye×St(Tr)	.	**		*



Appendix Figure 6-A: PCO ordinations of distance among centroids for macrofauna count data (MCD) from cage stations and reference stations: representing 4 year windows for periods: a) T0 – T3, and b) T1 – T4 (correspond to same windows for MCD in Appendix Table 6-C). Showing substantial but differing change at Cage stations compared with minimal change at Reference sites.



Appendix Figure 6-B: As for Figure 6-1, but representing 3 year windows for periods: a) T1 – T3, b) T2 – T4 and c) T3 – T5 (correspond to same windows for MCD in Appendix Table 6-C).



Appendix Figure 6-C: As for Figure 6-A, but representing differences according to HLTG and 4 year windows for periods: a) T2 – T5, b) T3 – T6 and c) T4 – T8 (correspond to same windows for MCD in Appendix Table 6-C).

CHAPTER 7

BENTHIC RECOVERY AND RE-IMPACT RESPONSES FROM SALMON FARM ENRICHMENT: IMPLICATIONS FOR FARM MANAGEMENT

Preface:

This chapter is an extension of the long-term recovery analysis provided in Chapter 6. It examines recovery rates at a comparable site, but more intensively and over a shorter period (2 years), and contrasts this with re-impact rates at an adjacent site that had been fallowed for eight years (described in Chapter 6). These findings have important implications for fallowing and mitigation strategies that are often employed to manage seabed effects, and therefore the sustainability finfish farming in some locations

At the time of thesis submission, this work was due to be submitted to a refereed journal and is presented below in pre-submission form. The proposed citation for the publication is:

Keeley N, Forrest B, MacLeod C. Benthic recovery and re-impact responses from salmon farm enrichment: implications for farm management.

7.1 Abstract

This paper describes a two-year study of spatial and temporal patterns and processes in the benthos in response to the removal (i.e. fallowing) of salmon cages from a sheltered coastal embayment, coupled with the simultaneous reintroduction of cages at an adjacent location. Significant recovery was evident at the fallowed site in the first six months; however, the macrofaunal assemblage remained impacted at the conclusion of the study. By comparison, the reintroduction of a fully operational farm overwhelmed the macrobenthic community within three months, with anoxic and near-azoic conditions developing. Both removal and reintroduction of the farms triggered alternating oscillations of geochemical and biological variables, which were attributed to effects on sediment chemistry from organic loading, 'boom and bust' cycles of opportunistic taxa in response to food supply, and the associated variations in metabolic potential. The study also revealed interesting spatial dynamics in the benthos and some useful indicators of different stages of recovery and re-impact. It is concluded that farm reintroductions should aim to gradually increase production; allowing time for the benthos to adapt to the additional organic flux, and be maintained at a level that avoids macrofaunal collapse. The sediments ability to cope with organic inputs from fish farming, and hence the duration of the recovery period, is contingent on the organic load in each farming cycle and the extent to which the sediment community is allowed to recover. Understanding the influence of each of these on sediment processes is important for sustainable long-term management of farming operations.

7.2 Introduction

Sea-cage aquaculture can result in high levels of localised benthic enrichment due to fluxes of organically rich biodeposits in the form of fish feed and faeces. As a result, mandatory seabed monitoring is common (Wilson et al. 2009) and the effects of operational farms are well described (e.g., Gowen & Bradbury 1987, Carter 2001, Buschmann et al. 2006). In most instances, these effects consist of extreme seabed enrichment characterised by sediment anoxia and a severely impoverished macrofauna community. Understanding the ability of the benthos to recover from effects of this magnitude is critical to determining the wider sustainability of marine farming activities. Estimates of recovery rates vary considerably, from 6 months (Ritz et al. 1989, Brooks et al. 2003) to five years or more (Brooks et al. 2004, Keeley et al. In Press.), and are highly environment and situation specific (Borja et al. 2010). Although complete recovery may take many years, a significant degree of biological and chemical remediation can occur in the first 6 to 24 months. A better understanding of recovery processes in these early stages is particularly important for farm management. In particular, a clear understanding of the recovery process is essential for evaluating the effectiveness of fallowing (periodic destocking) strategies that are often implemented to manage effects (Brooks et al. 2003, Macleod et al. 2006, Lin & Bailey-Brock 2008).

Fallowing (i.e. the temporary retirement of farmed areas) has two main purposes: i) to avoid significant environmental deterioration for the purposes of environmental compliance and to prevent conditions that may adversely affect fish health, and ii) to break the life cycle of parasites (e.g. sealice, Bron et al. 1993, Morton et al. 2005), especially in northern hemisphere countries. When implemented effectively, a fallowing strategy has the potential to increase long-term farm productivity and sustainability. However, the practice of fallowing requires that there be sufficient area for stock to be rotated, which in turn implies that a larger area of seabed may be impacted. This can be an important constraint, for example in areas where farms are situated close to habitats containing long-lived organisms that are more sensitive to enrichment (Hall-Spencer & Bamber 2007). The effectiveness and sustainability of fallowing practices is an important question for management, particularly where multiple fallowing cycles are employed and there may be potential for cumulative impact (Macleod et al. 2007) or where the system resilience may be compromised (Borja et al. 2010). One of the few studies undertaken in this area (Brooks et al. 2003) suggested that cumulative impacts may not be a problem. However, this finding needs to be considered in the context of site-specific

factors; for example: farming intensity, the level of impacts at the point of fallowing, the duration of the farming versus fallowed cycles, hydrodynamics and the endemic macrofauna composition can all influence recovery (Macleod et al. 2006, Macleod et al. 2007, Lin & Bailey-Brock 2008).

An improved understanding of sediment remediation processes will also contribute to our understanding of the benthic ecosystem's resilience to anthropogenic disturbance. Resilience can be defined as the properties that mediate the transition between different states (Gunderson 2000), and hence the changes that take place in the early stages of impact or recovery are particularly relevant. Although the fundamentals of successional response to disturbance, and in particular organic enrichment, have been well described (Pearson & Rosenberg 1978), there are a number of recognised exceptions to the Pearson and Rosenberg model (Maurer et al. 1993, Brooks et al. 2004, Keeley et al. 2013a). Of particular relevance is the fact that temporal succession during recovery does not necessarily mimic traditional patterns of spatial succession (Karakassis et al. 1999). Additionally, succession during recovery is seldom a mirror image of the temporal response to impact (Macleod et al. 2004a), and there is often a lag between the impact and recovery trajectories, termed 'hysteresis' (Borja et al. 2010, Verdonschot et al. 2013). The degree of hysteresis is therefore inversely related to level of resilience in the recovering system, which can be viewed as a type of 'memory' (Elliot et al, 2007, Verdonschot et al. 2013). It is therefore important to identify the general features and indicators for impact, recovery, and re-impact pathways independently.

In this study we compare two concurrent, relatively high frequency, medium-term (i.e. 2 year) studies at comparable sites to specifically identify key spatial and temporal patterns in the benthos in response to recovery and re-impact, and the rates at which they occur. The results are used to consider how initial levels of impact, or repeated impacts, might influence recovery, and what this means for fallowing and other management strategies , both in the medium-term and strategically looking to the future.

7.3 Methods

7.3.1 Study sites and sampling procedures

This study was conducted at two commercial Chinook salmon (*Oncorhynchus tshawytscha*) farms (Forsyth Bay = 'FOR' and Waihinau Bay = 'WAI', Figure 7-1) situated in sheltered embayment's in the outer reaches of the Marlborough Sounds, New Zealand. The sites had comparable sediments (average mud content = 78 to 91%), depth (28 to 35 m) and flow dynamics (mid-water mean current speed = 3 to 8 cm s^{-1}). Farm FOR was fallowed in 2001 after seven years of consistent and relatively intensive use, and remained unfarmed for the following eight years. Therefore, at the commencement of this study, the seabed at FOR was almost completely chemically and biologically remediated (Keeley et al. In Review). In December 2009, farming operations were relocated to the FOR site from the nearby WAI farm, which had been actively farmed for approximately 20 years. This provided the opportunity to conduct a concurrent study of the effects of fallowing (at WAI) and re-impact (at FOR). Average feed use at WAI over the 12 months preceding the shift was ~268 metric tonnes per month (mt $month^{-1}$), and feed inputs at FOR during the re-impact period equated to ~262 mt $month^{-1}$.

Both FOR and WAI were sampled immediately prior to cage relocation, then concurrently at 3-month intervals for the first 12 months after relocation, and then 6-monthly for the following year (i.e. at 0, 3, 6, 9, 12, 18 and 24 months). Sampling events are identified by the number of months ('M') post-fallowing at WAI or after re-instating at FOR, e.g. M0, M3, … M24 and are denoted by '×' on subplots in Figure 7-3. At FOR, samples were collected from: three cages stations (Cage1,2,3), five 'Gradient stations' at increasing distances from the farm along a north-western transect (i.e. 25 m, 50 m, 75 m, 100 m and 150 m, Figure 7-1), and two near-farm reference stations (Ref1 = 200 m, Ref2 = 400 m) with comparable depth and substrates. Sampling at WAI was conducted at two Cage stations (Cage1,2), three Gradient stations (50m, 100m and 150m), and at a reference station (Ref4) situated ~430 m away (Figure 7-1). A fourth reference station (Ref3) situated >4 km from both farms served as an additional 'far-field' reference for each.

Triplicate samples were collected at all stations using a Van-Veen grab, with water depth (Depth, m) and distance from farm (Distance, m) recorded. Each sample was analysed for grain size distribution (dried and analysed gravimetrically for size class fractions from silt-clay

through to gravel), organic matter content (%OM measured as % ash free dry weight; Luczak et al. 1997), total free sulphide (TFS, µM) and macrofaunal community composition. Sediment grain size and %OM measures were determined from sub-samples collected using a 5.5 cm diameter Perspex core, with the surface 30 mm kept for analysis. Total free sulphide was measured in the surface sediments (0-4.5 cm depth interval), with samples collected using a cut-off 5-cc plastic syringe, and analysed following the methods of Wildish et al. (1999). Macrofauna sub-samples were collected using a 130 mm diameter (0.0132 m^2) core (100 mm sediment depth). Macrofauna were sorted and enumerated to the lowest practicable level and their abundances recorded. Macrofauna count data were used to calculate total abundance (N), number of taxa (S), Pielou's evenness *(J')*, Shannon diversity *(H')* and the AZTI's Marine Biotic Index (AMBI, Borja et al. 2000), Benthic Quality Index (BQI, Rosenberg et al. 2004) and Multivariate AMBI (M-AMBI, Muxika et al. 2007). The M-AMBI calculations utilised the references conditions that were established for low flow sites in the Marlborough Sounds (refer Section 3.3.3). Qualitative assessments of sediment odour, Beggiatoa (bacterial mat) coverage and sediment out-gassing using pre-specified categories (as described in Keeley et al. 2012a) were also made at each station.

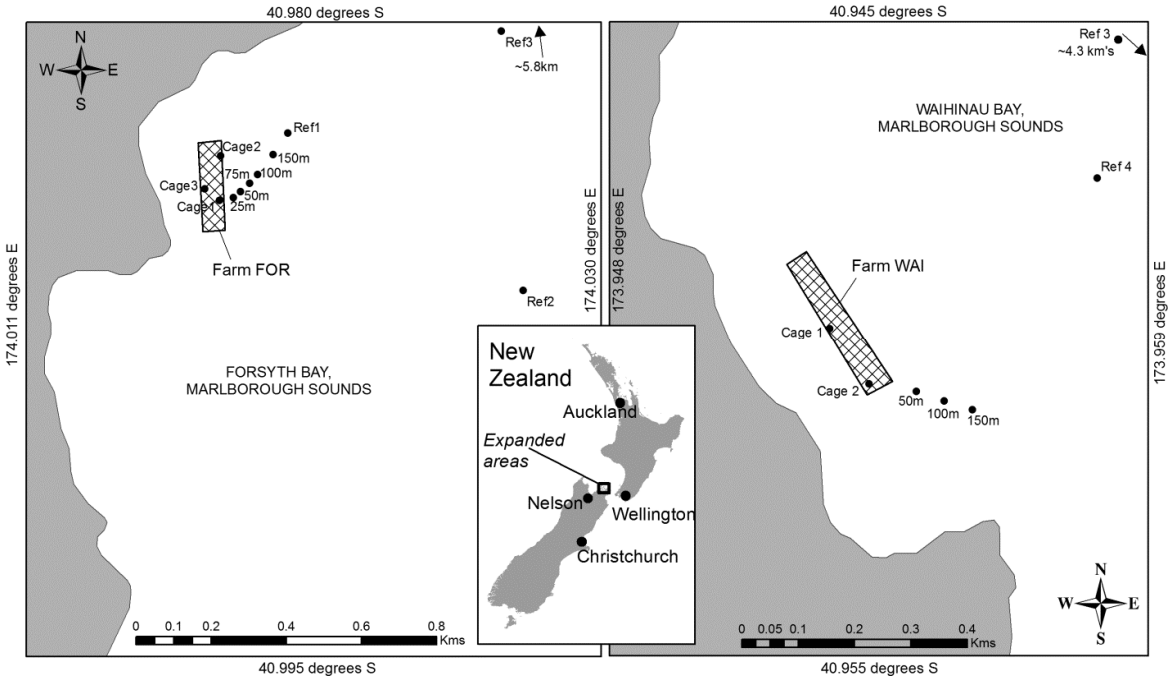


Figure 7-1: Location of study sites and sampling stations in relation to farms FOR (left) and WAI (right), Marlborough Sounds, New Zealand.

7.3.2 Data analysis and variable selection

Pearson correlation coefficients between variables were determined from replicate-level data with any strongly right-skewed variables (N and TFS) $\log_{10}$ transformed. Results are displayed using the 'ellipse' library (Murdoch & Chow 1996) in R (Figure 7-2). Several of the environmental indicators were highly correlated in both the recovery and re-impact datasets, which allowed selection of a sub-set of variables. Most of the diversity measures and biotic indices were positively correlated with each other (H', AMBI, BQI and M-AMBI) and negatively correlated with AMBI, with r-squared values >0.83. Log(N) was the most weakly correlated with other variables ($R^2 < 0.42$), and therefore may provide 'complementary' information. The set of variables that were selected for more detailed analysis was constrained to: %OM, log(TFS), log(N), S, and BQI, as well as overall enrichment stage (ES) which is a derivative of all of the physico-chemical and biological variables combined (see Keeley et al. 2012a). The results for the other variables (i.e. H', AMBI and M-AMBI) are presented in Appendix 7-A and 7-B.

Spatial and temporal patterns for individual parameters were interpolated into a grid using the Kriging method in Surfer 9, and displayed in 2-dimensional plots. The x-axis represents 'Time' in years relative to fallowing or re-impact (T0) and the y-axis represents 'Space' as distance from cages (m). Distances to Ref2, 3 and 4 were reduced for plotting to emphasise the changes that occurred over the first 200 m from the farm (changes beyond 200 m were negligible).

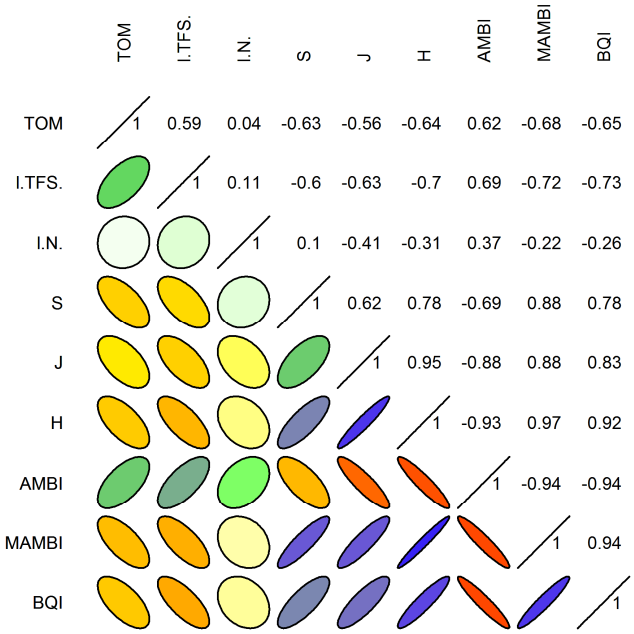


Figure 7-2: Pearson correlations between environmental variables. Ellipses indicate correlation strength (by degree of elongation and graduated colour from red = strongly negative to blue = strongly positive), numbers indicate coefficient rounded to 2 d.p.

7.3.3 Multivariate analysis of Cage and Reference stations

Species succession was described by using the SIMPER procedure to identify those taxa which contributed most to Bray-Curtis similarities within sample groups for each survey; groups consisted of sample replicates pooled within Cage or References stations. Bubble plots were generated to display temporal patterns in specific taxa using the ggplot() function in the ggplot2 library in R (R Development Core Team 2011), where the x-axis represents time (in months) and the y-axis represents individual species. The colour gradient of the symbols indicates the average (%) contribution to the groups' similarity (based on SIMPER analysis of fourth-root transformed data) and the bubble size indicates abundance for the given time. Taxa shown are restricted to those which contributed to the top 90% of the dissimilarity, and

the y-axis (i.e. taxa) was sorted (from top to bottom) according to their contribution to the groups' similarity, sequentially starting with M0 and progressing to M24. This creates a gradient of species succession; with those species that played an important role early in each study (i.e. either immediately post-fallowing or post farm re-introduction) placed at the top-left of the plot and those that contributed in the later stages (e.g. recolonization) toward the bottom-right. Taxa-specific 'Eco-Groups' (EG) are displayed where available, which are established classifications for species' sensitivity to organic enrichment that are used to calculate AMBI; these groups range from 'I' (very sensitive) to 'V' (first order opportunistic taxa) (refer Borja et al., 2000; Keeley et al., 2012b and http://ambi.azti.es/).

The temporal changes that occurred at both WAI and FOR were then compared using Multi-dimensional scaling (MDS, Kruskal & Wish 1978, Clarke 2006) in PRIMER v6. All macrofauna count data were square-root transformed to reduce the influence of highly abundant taxa and the station-averaged Bray-Curtis similarities of Cage and Reference stations over time (all months) were displayed in 2-dimensions. Additionally, the environmental data (%OM, log(TFS), log(N), S, H', AMBI and BQI) were normalised and MDS was used to display the similarities according to Euclidean Distances for Cage and Reference station through time.

7.4 Results

7.4.1 WAI site recovery

At the point of fallowing (M0), WAI was highly impacted immediately beneath the cages (i.e. the former cage site), with elevated TFS (average = 4335 µM), high %OM (average = 20%), low N (2 – 60, except one replicate which had 576 individuals), and, only a few (2 – 4) taxa (primarily *Capitella capitata* and nematodes, Figure 7-3a-d, 4).

Three months after fallowing the conditions at the Cage stations had deteriorated further; N and S were at near zero levels, TFS had increased and the biotic indices indicated extremely impacted conditions (Figure 7-3b-d & 7-4). Interestingly, %OM increased between M0 and M3 at one of the two cages stations, despite the fallowed status, before decreasing. Accordingly, overall Enrichment Stage (ES) indicated near-azoic conditions at M3 (average ES = 6.3). N increased sharply in the following two surveys, achieving a peak of >1700 individuals per core 9 months after fallowing. Over the same period, TFS reduced dramatically, but S remained very low (average S ≈ 6). The period when S was consistently low (M6 to M9), and total abundances were high, corresponded to consistently low BQI values indicating a 'bad' environmental status, according to the criteria of Rosenberg et al. (2004). Average overall ES reduced to 5.1 at M9 as the opportunistic taxa proliferated (Figure 7-3f).

Beyond 9 months, S progressively increased, achieving levels comparable to the Reference stations (S ≈ 18) by M24 (Figure 7-3d & 7-4). Average %OM remained elevated at the Cage stations until M12, and to a lesser extent M18, but decreased markedly between M18 and M24, at which point it was only slightly elevated (~6.5%, Figure 7-3a). After 12 months, N consistently decreased, but remained moderately elevated (average N = 397 individuals / core) at M24 (Figure 7-3d & 7-4). Marked improvements were evident in all other diversity measures and biotic indices between 12 and 24 months; however, only H' achieved levels indicative of Reference conditions by the conclusion of the study (Appendix 7-A). S, AMBI and M-AMBI all still indicated a moderately impacted state after 12 months, while the BQI indicated 'poor' benthic habitat quality (Appendix 7-A, Figure 7-3d,e). ES indicated progressive improvement in conditions from M3, achieving ES~3 at M24 (Figure 7-3f, 4)

The gradient sampling stations beyond the cage showed a similar temporal response pattern during recovery, but with an approximate 3 month lag. At 50 m, N was initially

elevated, but reduced during the first 6 months of recovery before peaking again at 12 to 18 months. Thus, both the dip in N (evident as a collapse beneath the Cages at M3) and the subsequent peak occurred 3 to 6 months later than beneath the cages (Figure 7-3c). S, AMBI and M-AMBI also all indicated a temporary deterioration in conditions at the near-farm (50 m) stations at 6 and 9 months before improving steadily between M12 and M24. ES indicated consistent moderate levels of enrichment (ES ≈ 3) 50 m away for the first 12 months before reducing to near-background levels at M24 (average ES = 2.5). Further way from the cages at the 100 m and 150 m stations most of the biotic indices (e.g. H', AMBI) returned to near background by M12 to M18. Overall ES was very slightly elevated (ES ~0.3 > Reference) at 100 m until M24. The levels of enrichment encountered at 50 m distance at M0 were approximately equivalent to those that were observed at the Cage stations after 24 months of fallowing.

The initial reduction in total N at M3 at Cage stations was primarily due to a reduction in the abundance of the opportunistic polychaete *Capitella capitata* (EG V), and to a lesser extent, nematodes and amphipods (Figure 7-5). The subsequent abrupt increase in N at 6 months was due to large increases in *C. capitata* abundances and small increases in nematode worms, dorvilleid polychaetes (both EG II second order opportunists) and the polychaete *Neanthes circognatha* (EG III). *C. capitata* densities continued to increase at 9 months, while the other three species reduced in abundance.

After M9, *C. capitata* abundances strongly decreased whilst three second-order opportunists (nematodes and dorvilleid polychaetes) increased and remained dominant until M24. Several other less impact-tolerant taxa became important components of the assemblage at M12 (the small bivalve *Theora lubrica,* amphipods and some EG II and III polychaetes: *Prionospio multicristata, Boccardia acus,* and representatives of the families Glyceridae and Nereidae). Many of these taxa had declined in abundance three months later; the exceptions being *T. lubrica* and *P. multicristata,* which remained numerically important at M24. The main changes between 18 months and the final survey (at 24 months) were due to the third consecutive large decrease in *C. capitata* abundance, and relatively minor changes in abundances of several taxa known to be sensitive and / or indifferent to enrichment (i.e. EG I or II), e.g. the polychaetes *Boccardia* sp., *Heteromastus filiformis*, and the families Maldanidae, Paraonidae, Lumbrineridae, and cumacea (Figure 7-5).

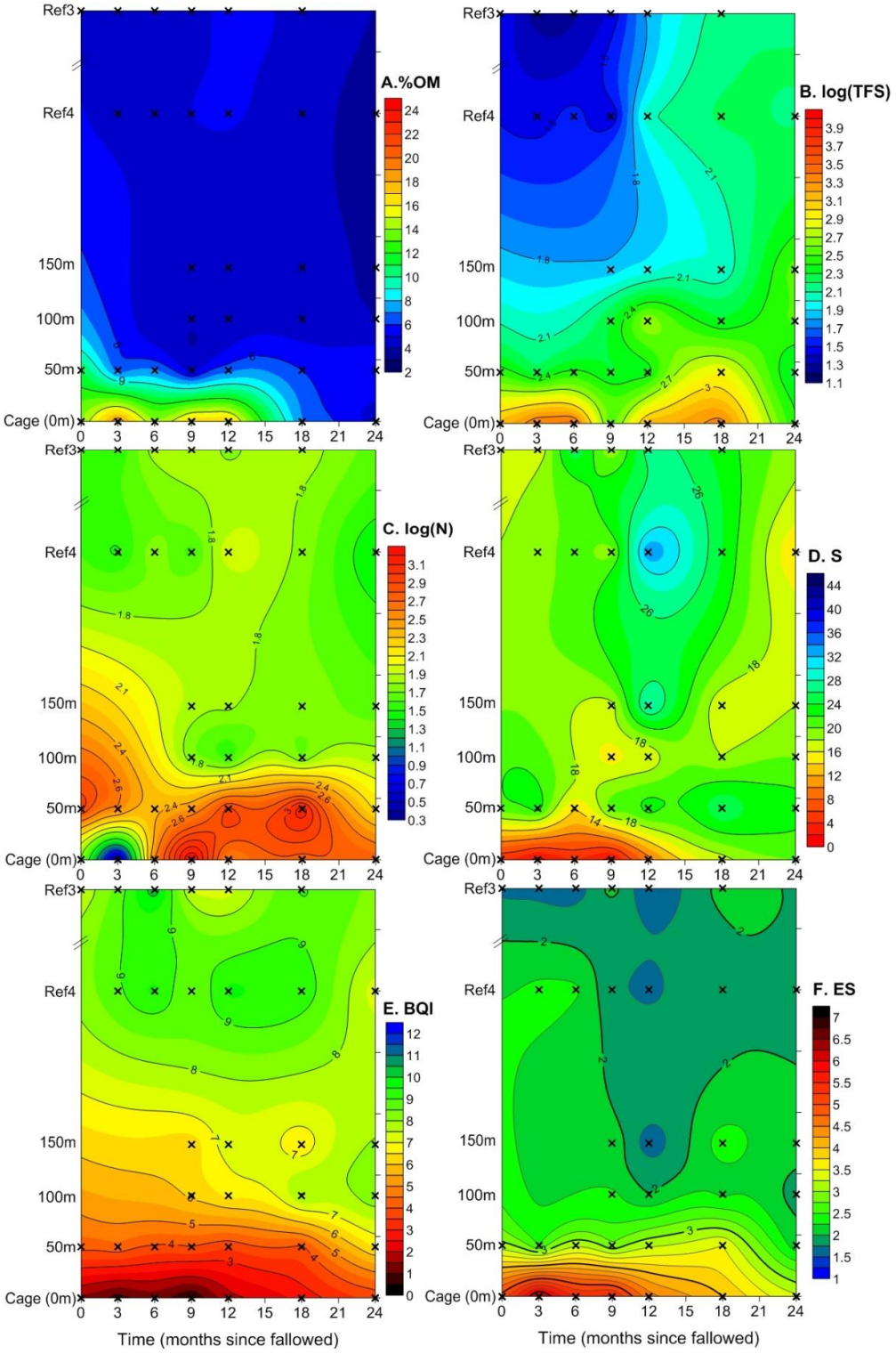


Figure 7-3: Changes in %OM, log(TFS), log(N), S, and BQI with space and time during recovery at WAI. Time is given in months relative to when the cages were removed (M0) and space is the square of distance (m) from the cages. Crosses ('×') denote sampling events.

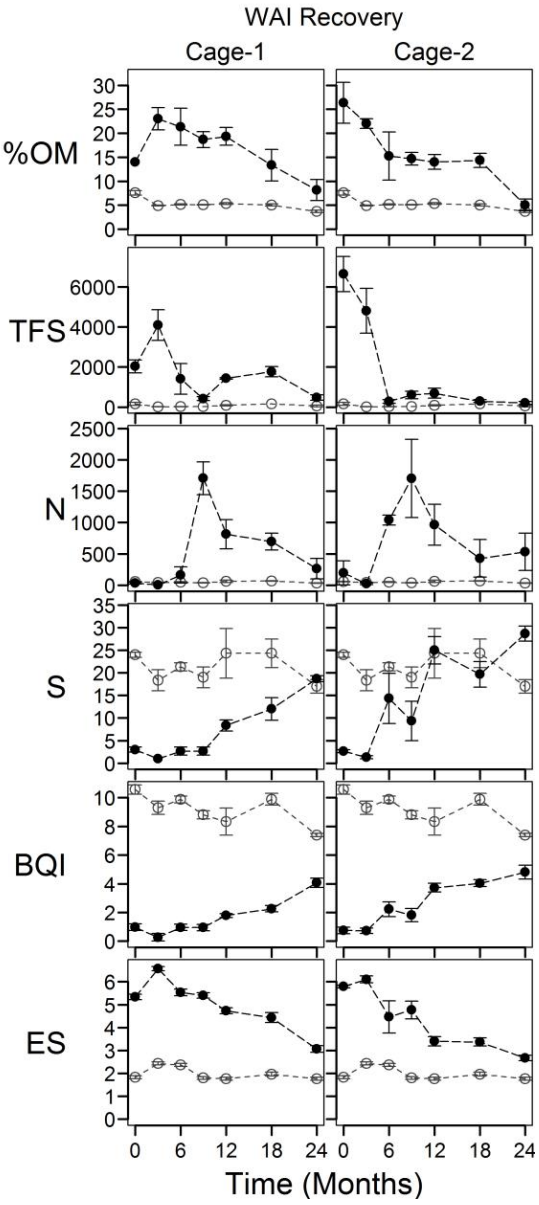


Figure 7-4: Scatterplots of average %OM, TFS, N S, BQI and ES at WAI recovery at Cage stations 1 and 2 (black dots). Open circles indicate mean values for Reference stations. Error bars represent 1SE.

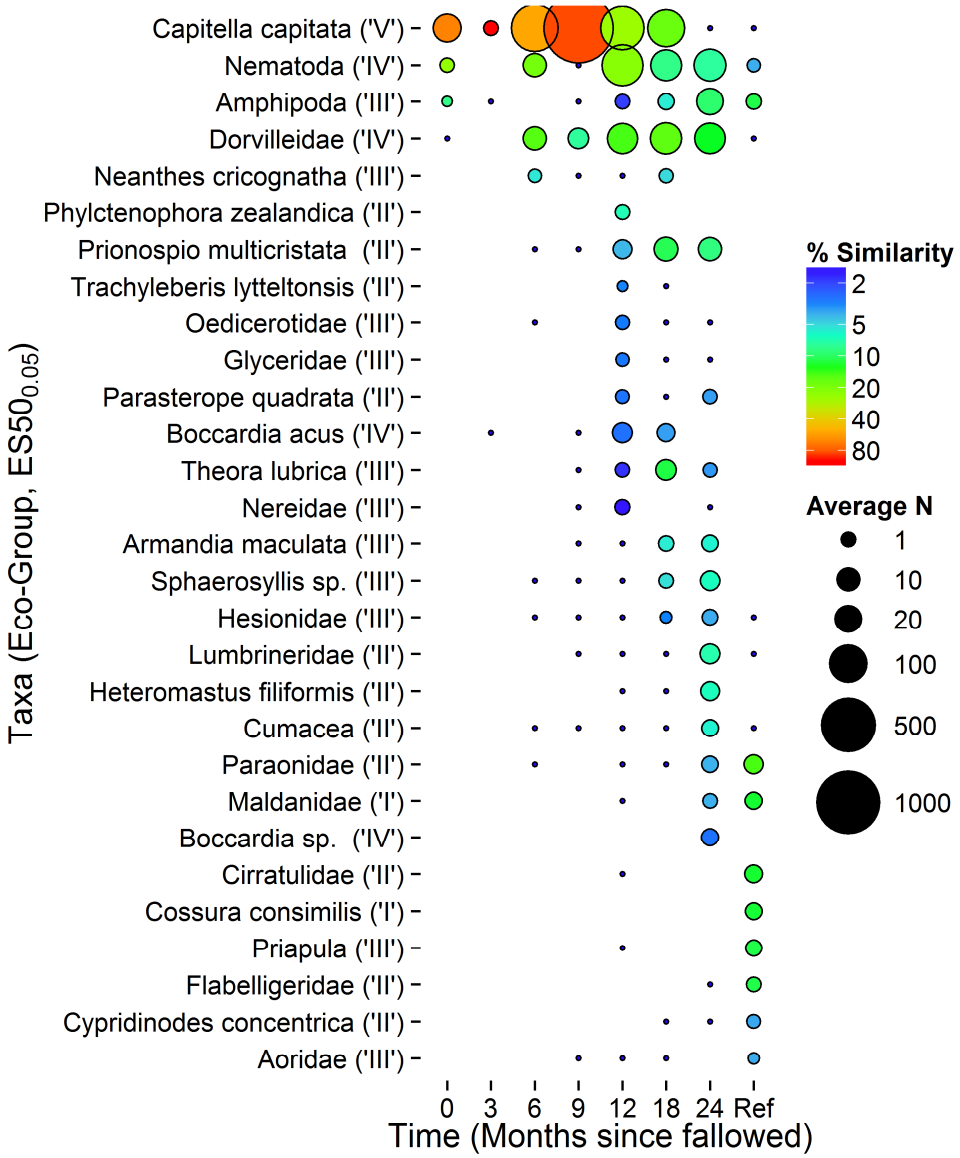


Figure 7-5: Bubble plot of WAI short-term recovery at Cage stations (combined) based on SIMPER analysis of macrofauna count data. Species shown are those that contributed to the top 90 % of the groups' similarity and are sorted (from top to bottom) according to their relative contribution (% similarity indicated by colour gradient). Bubble size indicates (fourth-root scaled) average total abundance (N). Ref = reference stations at M24. Bracketed values indicate previously established Eco-Group (Borja et al., 2000) values for each taxon. Small blue dots indicate taxa that were present, but did not contribute significantly to the similarity.

7.4.2 FOR site re-impact

The benthic response beneath the cages during re-impact at FOR was characterised by a rapid deterioration in all indicator variables (Figure 7-6a-f). Three months after reinstatement the seabed had changed from a near-natural state (average N = 54, average S = 19.4, ES ≈ 2) to a severely impoverished macrofauna (average N = 16, S = 3, ES ≈ 6; Figure 7-6c,d,f and 7), with some replicates containing no macrofauna. Over the same period, there was a small increase in TFS, which continued to increase over the following 6 months, peaking at 4000 – 5000 µM 9 months after the farm was reinstated (Figure 7-6b and 7-7).

N remained low for the first 6 months before a substantial peak, driven predominantly by *C. capitata,* which occurred 12 months after reinstatement at all three Cage stations (average N = 2,380, Figure 7-6c and 7-7). This peak was still evident after 18 months. At the conclusion of the study (i.e. after 24 months) the peak of opportunists had diminished (average N = 276), with three of the nine samples containing only 5 to 10 individuals per core. After the initial decline at M3, S continued to decline at M3 and M6 and remained very low (average of 3 to 4 taxa / core) for the remainder of the study (Figure 7-6d and 7-7). BQI and AMBI also indicated a highly impacted state (1 – 1.5 and > 5.8, respectively) from 12 - 24 months post-reinstatement. TFS peaked again, strongly in the last survey (M24), after N had diminished (Figure 7-6b). %OM was positively correlated with TFS, peaking initially at M9 to M12 and again at 24 months (~20% w/w, Figure 7-6a). Elevated levels of %OM were highly localised, not extending much beyond 25 m from the farm. ES reduced slightly after 12 to 18 months in accordance with the temporary re-establishment of opportunistic taxa, but increased to >ES 6 again at M24 when the macrofauna collapsed.

A peak in N was also evident at the Gradient stations, but diminished with increasing distance (from 25 m to 100 m stations) and occurred 3 months earlier than beneath the Cages (i.e. after 9 months Figure 7-6c). This small peak in N was observed as far out as the 150 m station. At 25 m, the peak in abundance was still evident at the conclusion of the study, but further away (i.e. at 50 m and 75 m) abundances subsided and were comparable to background levels at M18. S was reduced by approximately 50 % when N peaked at the 25 m station (at M9), but had returned to reference levels by M12 (Figure 7-6d). At the 50 m and 75 m stations, S remained moderately supressed (~15 taxa / core) at 18 and 24 months. Similar patterns were evident in the biotic indices (e.g. AMBI and BQI, Appendix 7-B and Figure 7-6e,f)

at the 25 m to 75 m stations – all of which indicated significant, progressive deterioration over the first 9 months followed by a low level of improvement.

The abrupt deterioration in conditions beneath the Cages in the first 3 months after restocking was associated with the disappearance of virtually all of the taxa that were present at M0 (Figure 7-8). The most notable reductions were (in reducing order of importance according to SIMPER analysis): amphipods, *Prionospio aucklandica*, cumaceans, *Tanaid* sp., Paraonidae, Cirratulidae, Lumbrineridae, *Myriochele* sp., *Theora lubrica*, ostracods, Asellota, *Cossura consimilis*, and *Heteromastus filiformis* (Figure 7-8). At M3, small increases were observed in dorvilleid, *Prionospio yuriel,* and *C. capitata* polychaetes appeared for the first time. Beyond 3 months, differences between surveys beneath the cages were strongly dominated by large fluctuations in abundances of *C. capitata* – increasing initially from M3 to M6 and peaking in abundance after M12, before undergoing large declines M18 and M24. After the initial increase at M3, *P. yuriel* no longer featured in the Cage communities, and abundances of dorvilleid polychaetes remained relatively low and fluctuated between surveys. Nematodes became numerically important temporarily at M18, when *C. capitata* were declining (post-peak abundance), but still abundant.

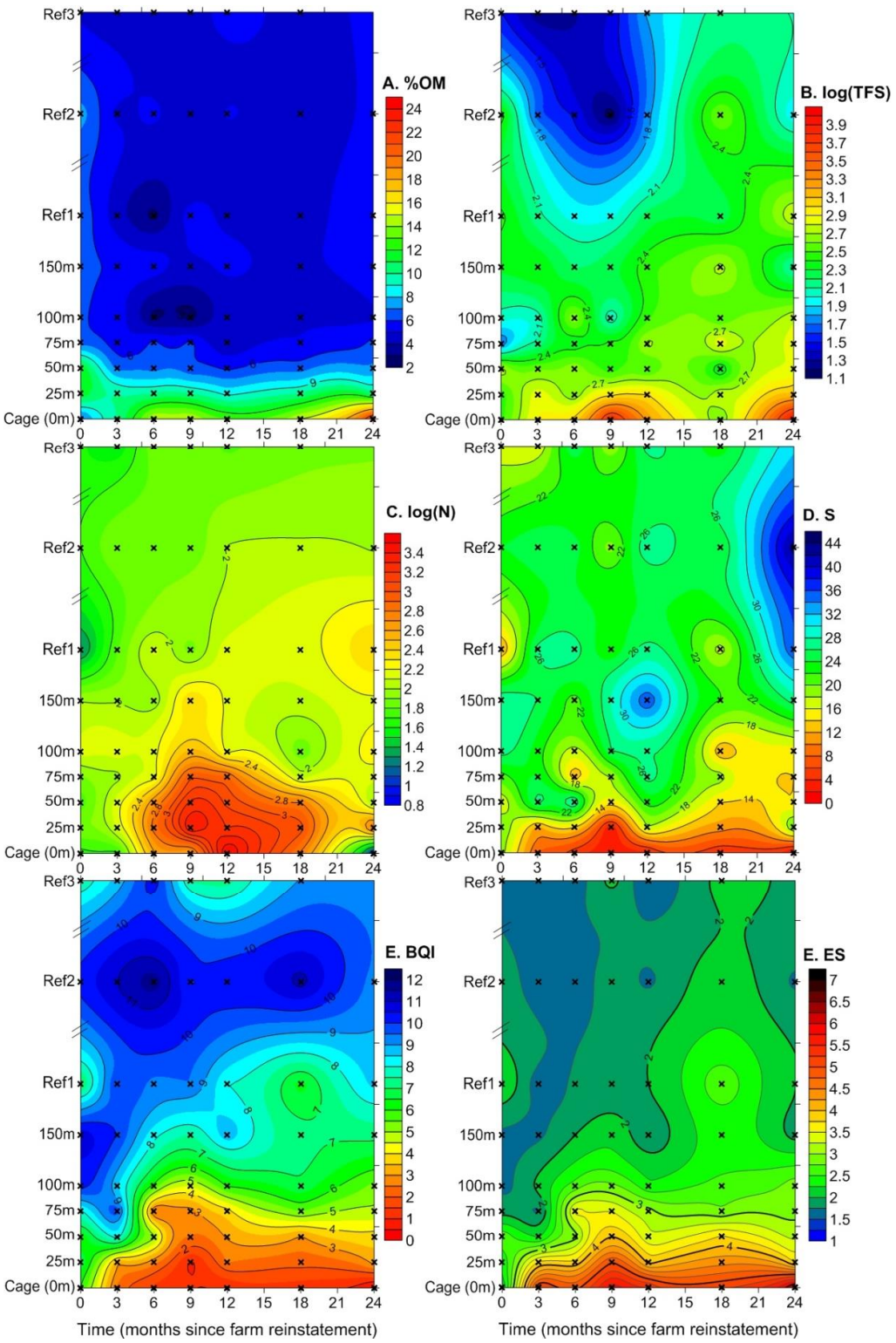


Figure 7-6: Changes in %OM, log(TFS), log(N), S, and BQI with space and time in response to farm reinstatement at FOR. Time is given in months relative to when the cages were reinstated (M0) and space is represented as the square of distance (m) from the cages.

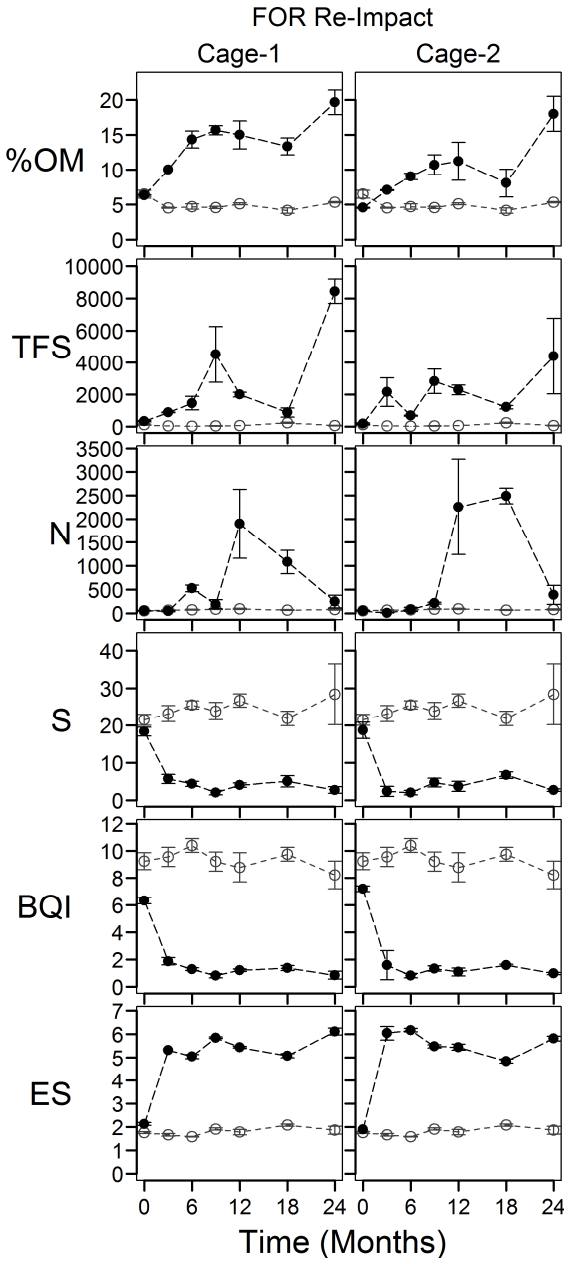


Figure 7-7: Scatterplots of average %OM, TFS, N S, BQI and ES at FOR re-impact at Cage stations 1 and 2 (black dots). Open circles indicate mean values for Reference stations. Error bars represent 1SE.

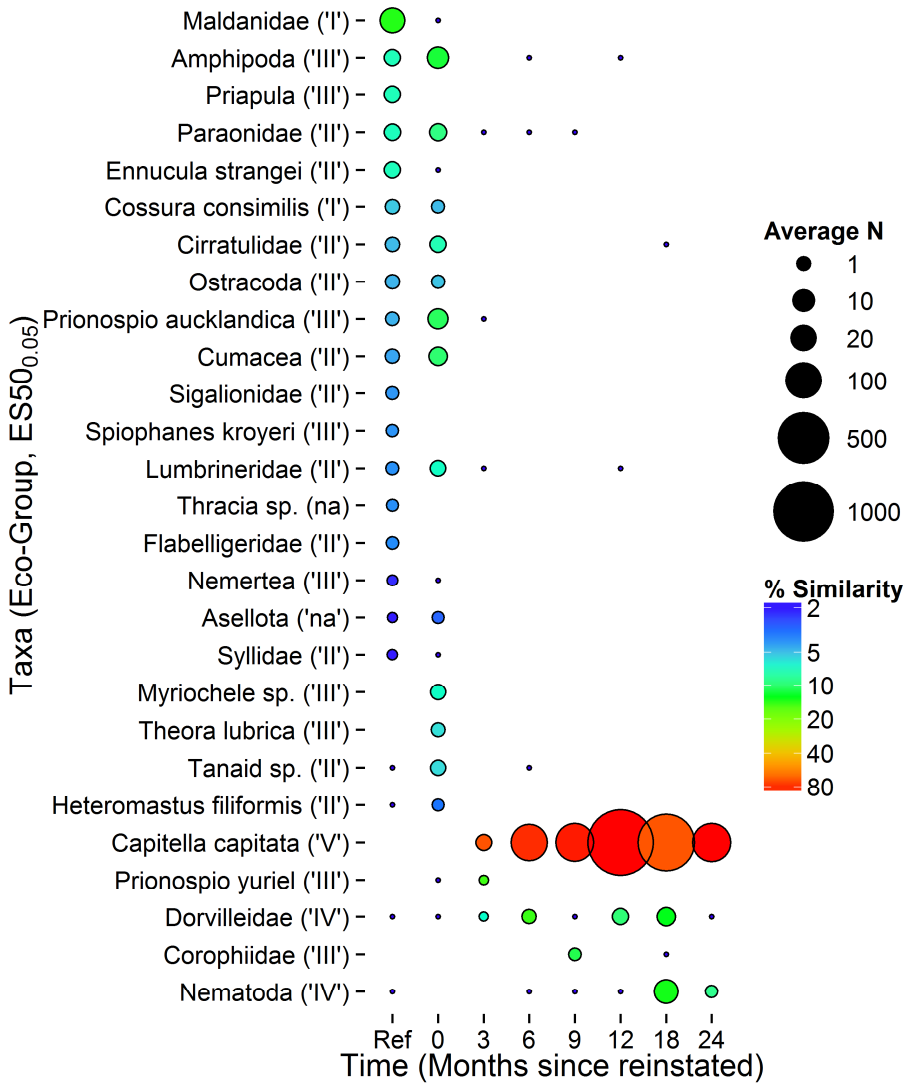


Figure 7-8: As for Figure 7-5, but using FOR re-impact Cage station data (M0-M24); Ref = Ref1 and 2 combined at M0.

7.4.3 Comparison of recovery and re-impact trajectories in benthic assemblages

The macrofaunal assemblages at the Cage stations after the first 3 months of recovery and re-impact were surprisingly similar; in both situations their biogeochemical and ecological conditions indicated a highly impacted state (Figure 7-9a,b). However, progression to this state from M0 was far more dramatic under the re-impact scenario, where the start point was

near-natural conditions, whereas the deterioration under the recovery scenario was a relatively small shift due to the highly impacted initial state. The recovery and re-impact stations also behaved similarly between M3 and M6, where the levels of TFS and %OM were similarly high, opportunistic taxa proliferated, and the benthos was otherwise impoverished. After 6 months, conditions at the re-impact stations remained highly impacted and generally comparable to conditions at the recovery site prior to fallowing. Conditions at the recovering site progressively improved from 9 months onwards, increasing in similarity to the Reference stations, but still remaining distinct (and impacted) at 24 months.

There was very good agreement between the Bray-Curtis similarities of macrofauna count data and the Euclidean Distances based on the suite of environmental indicators (Figure 7-9a,b). Variation within the Reference stations between surveys was small in comparison to the changes through time at the Cage stations. Differences between the two reference stations (i.e., Ref1 near to FOR and Ref4 nearer to WAI) were also consistently relatively small.

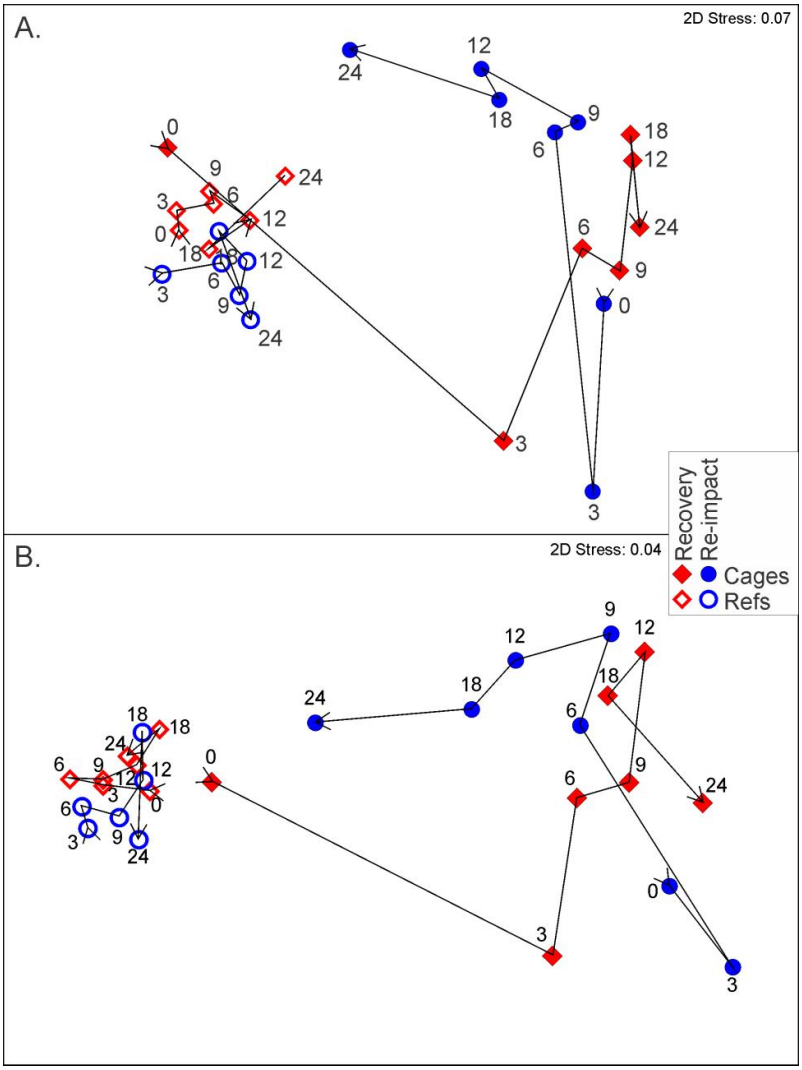


Figure 7-9: MDS ordinations of FOR and WAI medium-term time-series data for Cage and Reference sites (FOR = Ref1, WAI = Ref4), based on A. Bray-Curtis similarities of site-averaged, square-root transformed macrofauna count data; and B. Euclidean distances of site-averaged normalised environmental data (variables include: %OM, log(TFS), log(N), S, H', AMBI and BQI).

7.5 Discussion

7.5.1 Recovery processes

Medium-term recovery processes can be defined by a series of key responses. Recovery at WAI was most significant in the first six months, with an initial increase followed by a rapid decline in TFS concentrations over this period. In the ensuing three months there were marked increases in the abundance of opportunistic taxa. The number of taxa (S) remained suppressed for the first six months but then steadily increased as the opportunists declined. S returned to levels close to that of the reference stations after 2 years, however, the community was still clearly impacted, with second-order opportunists and enrichment tolerant taxa dominating, and lacking several taxa that were numerically important at the reference station. The resulting overall enrichment stage (ES) remained clearly higher than for the reference areas (ES 2.8 compared with ES 1.7 for the reference site). This is consistent with a number of other studies which have shown short-medium-term recovery in some variables (particularly geochemical) but distinct differences in ecological composition after similar timeframes (Karakassis et al. 1999, Macleod et al. 2004c, Villnas et al. 2011). Such large shifts in S, N and in taxa composition were well captured by the diversity measures and biotic indices, which all indicated highly impacted conditions for the first year and moderately impacted conditions thereafter.

The finding that full biological remediation at WAI was still not achieved after two years was not unexpected, as significant compositional differences can exist in the macrofauna for 5 or more years following a highly impacted state in similar low flow environments (Keeley et al. In Review). For this reason, formal criteria for the assessment of the recovery end-point described in Chapter 6, were not applied in this study. Brooks et al. (2004) define biological recovery as occurring when the dominant taxa (i.e. those taxa which comprise > 1 % by number) found at a reference site are present. Whereas here, only 39 % (s.e. = 2 %) of the dominant reference station taxa were present at the conclusion of the study. In contrast, the criteria for chemical remediation (Brooks et al. 2004) were met after approximately 18 months; at which point TFS was decreasing, redox levels were increasing and more than half the reference area taxa had been established. Hence, this study revealed early chemical remediation relative to biological remediation, as has been observed elsewhere (Macleod et al. 2004c, Macleod et al. 2006, Keeley et al. In Review).

7.5.2 Temporal dynamics during recovery and re-impact

Dynamic relationships between the biological and geochemical measures of recovery and re-impact were most evident as alternate oscillations between TFS and N - or more specifically, abundances of first-order opportunistic taxa, especially *Capitella capitata* and nematodes. In both the re-impact and recovery datasets, an initial peak in TFS was followed by a subsequent peak in N (3 to 6 months later), which in turn corresponded to a trough in TFS, and as N declined, a second peak in TFS was observed (a stylised representation of these general patterns is shown in Figure 7-10). In the case of the re-impact scenario, the first peak in TFS is presumably the initial response to farm reinstatement and organic inputs causing a rapid increase in %OM, which alters sediment chemistry and elevates TFS. When this occurs in relatively unimpacted sediments, the natural benthos may be poorly equipped to respond (Macleod et al. 2007) and sulphide-sensitive taxa (which may comprise the majority) will be eliminated, whilst enrichment tolerant taxa (characteristically smaller and with a shorter-life cycle) proliferate (Hargrave et al. 2008).

If sulphide tolerant taxa, such as *C. capitata* and nematodes, are not a pre-existing component of the macrofauna, then there may be a lag in their response (Gremare et al. 1989), during which time the sediment can be effectively devoid of macrofauna, such as was observed here. The establishment and subsequent proliferation of *C. capitata* in response to enrichment is not dependent on their presence at the time of the farm reintroduction because elevated TFS is a known settlement cue for the species (Cuomo 1985). However, rapid recolonisation would be facilitated by an established reservoir of the species allowing immediate, local reproduction.

The peaks and collapses of the opportunistic taxa illustrate characteristic 'boom and bust' cycles typical of r-strategy species, exacerbated by excess organic accumulation resulting in extreme hypoxia / anoxia. The state of the macrofaunal assemblage has previously been linked to the rate of supply of organic matter, and associated TFS concentrations (Tenore & Chesney 1985, Brooks & Mahnken 2003a, Hargrave et al. 2008). Being a first-order opportunist, *C. capitata*, responds rapidly to increased food availability and subsequent diminution of food can cause a collapse in the population, often on 8 to 10 week cycles (Chesney & Tenore 1985, Gremare et al. 1989). Such an 'overshoot of their carrying capacity' is thought to result from i) changes in physical factors, ii) reduction in availability of resources, or iii) competition for food (Gremare et al. 1989). These factors may explain the fluctuations

post-fallowing at WAI, as the pool of organic matter is consumed, at which point, competition from second-order opportunists may also become important.

However, the same factors do not explain the re-impact situation at FOR where there was a substantial and consistent organic flux. The reduction in TFS at 12-18 months may be explained by fauna-mediated oxygenation of the sediments and organic matter decomposition (Heilskov & Holmer 2001, Braeckman et al. 2010). Although *C. capitata* was described by Heilskov et al. (2006) as a relatively poor irrigator, and therefore mineralizer, of sediments, that finding was based on relatively low densities, and therefore, low metabolic potential. The metabolic rate of Capitella has been estimated at 12 mmol Total CO_2 m^{-2} day^{-1} for 10,000 individuals, whereas, a much larger Nereid polycheate species can have a metabolic rate almost an order of magnitude grater (Heilskov & Holmer 2001). However, capitellid densities in this study (1000 to 2500 per core) were approximately 2-3 orders of magnitude higher than is typical for large errant polychaetes (<10 per core), and can be as much as four orders of magnitude higher (Keeley et al. 2012b, Keeley et al. 2013a). Additionally, Heilskov's study considered the ability of capitellids to actively irrigate the sediments, but it did not account for a number of other factors that can promote mineralization of organic matter when densities are very high. For example, there is likely to be significant passive transfer of overlying waters and microbial communities through the intensive burrow networks, and the mobilisation of sediments through ingestion. It is therefore reasonable to conclude that opportunistic macrofauna when at very high densities can play a significant role in waste metabolisation and associated chemical remediation. Nonetheless, with an ongoing flux of organic matter at FOR, sediment anoxia and collapse of the macrofauna eventually occurred (i.e. after 24 months). These conditions may supress future colonization events, leading to prolonged anoxia and organic accumulation. In the absence of a functioning and prolific macrofauna, benthic metabolism would be restricted to bacterial and anaerobic processes (Hargrave et al. 2008), which may explain the sharp increase in TFS at FOR from 18-24 months.

It was interesting to note that the abrupt removal of the WAI farm also set up an analogous set of oscillations in key biological and physico-chemical variables. Such oscillations between TFS and total abundance may go some way to explaining the frequently poor negative linear relationship between the two variables (Figure 7-2, Hargrave et al. 2008, Keeley et al. 2013a); as there can also be periods when both N and TFS are increasing. A better understanding of the role of the dynamic and complex bacterial communities that are an

important but poorly understood component of the benthic ecology (Bissett et al. 2007) may help explain these anomalies in the temporal cycles. It is likely that temperature / season also played a role by influencing metabolic rates (including that of the bacterial communities) and near-bottom oxygen levels. However, the patterns observed (summarised in Figure 7.10) are clearly dominated by the large post-disturbance response that would like override any seasonal pattern in the early stages at least, and did not seem to support a clear seasonal cycle.

Compared to recovery, the re-impact trajectory was steep, achieving a highly impacted state (near-azoic, ES 6 – 7) from near-natural conditions within three months (Figure 7-10). Whereas, the same level of recovery (i.e. from ES6 to natural) did not occur within the two year timeframe of the study, and a long-term study conducted at the same FOR site indicated that recovery was achieved after ~ 5 years (Keeley et al. In Review). Such hysteresis has been described for a range of environments, including rivers, lakes, estuaries and coasts (Borja et al. 2010, Verdonschot et al. 2013). However, the temporal model described for present study differs markedly from that proposed by Borja et al. (2010), which describes hysteresis (and resilience) in relation to a gradient of on-going pressure (i.e. where the pressure is ramped up or down), whereas in the case of salmon farm fallowing, the pressure changes abruptly (i.e. it is either constantly on (when the farm is present) or off (when the farm is removed). Interestingly, the level of organic enrichment (as assessed by ES) decreased periodically as opportunistic taxa became established and proliferated, before deteriorating again at the conclusion of the two year study, when the opportunistic taxa collapsed. It is conceivable that N may have peaked again after the conclusion of the study, but it seems unlikely in the presence of sustained high levels of organic flux. This suggests that the rate of organic flux (and accordingly feed use and farming intensity) was more than the assimilative capacity of the sediments. The average feed use over the period of occupation equated to a depositional flux of approximately 7 – 8 kg solids m^{-2} $year^{-1}$ beneath the cage (determined from depositional modelling (DEPOMOD), Cromey et al. 2002a, Keeley et al. 2013b). This assessment is consistent with the recently proposed threshold for the maintenance of ES5 conditions at low flow sites of ~ 6 kg solids m^{-2} $year^{-1}$ (Keeley et al. 2013b) and would suggest that the level of farming was unsustainable.

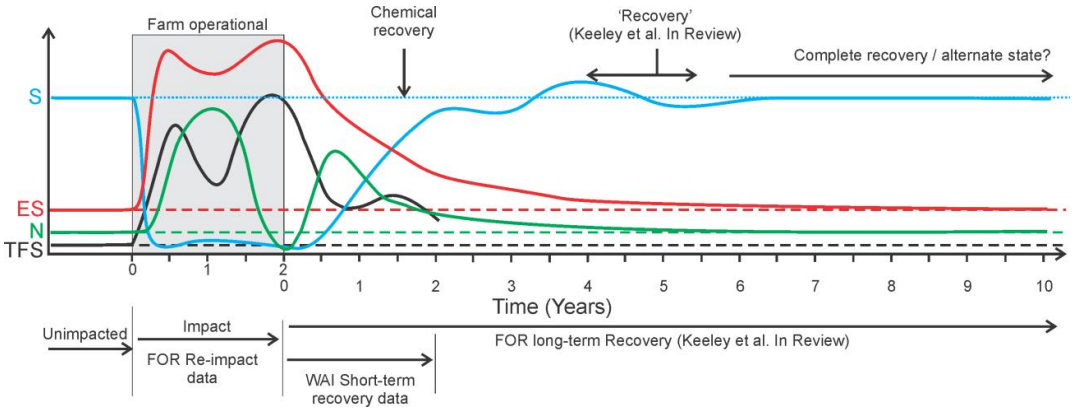


Figure 7-10: Stylized depiction of main features observed during impact and recovery from extreme enrichment. Integrates the general trends evident in the recovery and impact datasets (summarised from Figures 4 & 7) and from the long-term recovery patterns described in Chapter 6. S = No. taxa, N = total abundance, TFS = total free sulphides and ES = overall Enrichment Stage.

7.5.3 Spatial dynamics during recovery and re-impact

It was also apparent from this study that the peak in opportunists can shift in space as well as time in response to significant organic inputs, on a scale of 10's of meters. When the farm was operational at WAI, the zone of peak abundance occurred beyond the perimeter of the cages (25 to 50 m away), but shifted in to the centre of the farm when the inputs ceased and enrichment levels subsided. As recovery progressed (in this case, after 18 months), %OM reduced beneath where the cages were situated and the peak in total abundance shifted outward again to the perimeter of the site (25 – 50 m away). A comparable pattern was evident in a study of long-term recovery at FOR, where the peak in %OM shifted out to 25 m after two years and the peak in total abundance also occurred at the same distance two years later (four years after fallowing, Keeley et al. In Review). A similar situation was observed during re-impact, where the proliferation of opportunistic species initially occurred on the periphery of the cage site, before migrating in to the most impacted region approximately three months later. Such shifts in the high-density zone of opportunists close to the farm emphasises the spatially and temporally dynamic nature of the benthos and highlights the need for coupled at-source (e.g. beneath cage) and near-source (e.g. 50 m) sampling. This has implications for monitoring strategies that target sampling at a single distance from the farm (e.g. AZE, ASC 2012).

This study also revealed some interesting wider spatial patterns, whereby taxa richness, peaked both away from the farm in the outer reaches of obvious enrichment (i.e. 75 m to 100 m), and later in time, as the overall enrichment level subsided. A similar pattern was observed in the long-term analysis of the FOR site, where taxa richness became elevated approximately 100 m away from the farm, but in that case after 3 – 4 years (Keeley et al. In Review). Such observations are consistent with the established 'intermediate disturbance hypothesis', which predicts that the highest diversity will be found at intermediate levels of disturbance (Petraitis et al. 1989), analogous to the 'transition zone' described for organic enrichment gradients (Pearson & Rosenberg 1978). In this case, the outer region of the benthic footprint was characterised by ES2.5-3 conditions, which represents a zone of mild enrichment or 'enhancement' where taxa richness can be elevated, while sediment chemistry is not necessarily affected (Keeley et al. 2012b, Keeley et al. 2013a). This benthic state may be subjectively viewed as being either a positive or a negative effect, and given that the affected area is potentially larger than the area of 'severe enrichment', it should be a consideration in any broader environmental impact assessment (EIA).

7.5.4 Biological indicators

Comparisons of the prevalence of individual taxa during different stages of recovery as compared to re-impact were made difficult by the macrofaunal collapse that occurred at the re-impact site within the first 3 months. Two taxa were able to withstand the reintroduction of the farm (dorvilleids and *C. capitata*) both of which are first or second-order opportunistic polychaetes, and obviously hardy and indicative of high levels of enrichment. *Prionospio yuriel* proved to be moderately tolerant of organic enrichment, but it disappeared with the onset of sediment anoxia. Nematodes appeared to replace *C. capitata* towards the end of the 24 month cycle and as such featured at the most impacted end of the successional gradient. Succession among these taxa under degenerating conditions appears to be: dorvilleids → capitellids → nematodes → azoic. In the case of recovery the same taxa dominated from 6 months onwards, however *C. capitata* was the earliest to colonise and to subsequently die back. In the case of nematodes at least, the slightly delayed response in both degrading and recovery sediments may be related to their small size (meiofauna) and ability to occupy interstitial spaces (Sutherland et al. 2007); i.e. high densities of capitellids may in fact be

conditioning sediments and providing direct habitat, and as such there may be some degree of co-facilitation.

Diversity improved considerably after 12 months as several enrichment-tolerant and second order opportunistic taxa (EG III and IV, Borja et al. 2000) became established. These are all good indicators of the early stages of recovery. The presence of Maldanidae at the late stages of recovery when conditions were still moderately impacted raises questions over its present classification as being 'sensitive to enrichment' (Keeley et al. 2012b), suggesting it may be more appropriately classified as EG II (i.e., indifferent to enrichment, Borja et al. 2000). However, this decrease may also be a function of endemic and / or species-specific differences existing within what is a relatively high-level taxonomic grouping.

7.5.5 Implications for monitoring and management strategies

The fact that the impact/ re-impact pathway is considerably shorter than the recovery pathway (i.e. system hysteresis) has implications for management and sustainability of rotational fallowing strategies. Clearly, it may be impractical to move cages every three months to avoid sediment anoxia. Moreover, many alternate sites would be needed to move cages onto while the original sites were recovering, leading to a more extensive overall effects 'footprint' and creating conflict with other resource users. The sustainability of the level of farming may also be influenced by the way a farm is introduced to a site. In the present case, the farm was reintroduced in a fully operational state (i.e. fully stocked with large fish), as opposed to progressively with a relatively low initial biomass of smolts. This is likely to have contributed to the abrupt deterioration in conditions, as there was no lead-in time to allow the macrofauna to adapt and respond to the additional inputs. A different impact trajectory may have resulted if the farming intensity was progressively introduced over a period of ca. 6 to 12 months. Equally, the need for 'preconditioning' could be used as an argument for reintroducing farms to fallowed sites before recovery is complete, when the macrofauna assemblage still contains opportunistic taxa. However, this would only be true if there were no other residual effects in the sediments that predisposed it to becoming quickly re-impacted, for example, pockets of recalcitrant material or a shallow redox potential discontinuity (RPD) layer.

Where it can be assumed that residual effects are unimportant (i.e. system resilience has not been compromised) and the impact pathway (as indicated by ES, Figure 7-10) remains unchanged upon subsequent reinstatements, then the level of impact at reinstatement would likely increase initially, but remain relatively constant for subsequent fallowing/farming cycles (Figure 7-11-A). Conceivably, this situation would enable farms to consistently operate within benthic environmental quality standards (EQS), which is a common requirement for fish farms internationally (Wilson et al. 2009). However, if the system has compromised resilience at the point of reintroduction and the rate of re-impact increases, then a degenerative profile may develop (Figure 7-11-B). Once the population of opportunist's collapses, the potential for benthic metabolism (and therefore assimilation) also diminishes, and organic matter will tend to accumulate. Under these conditions, the recovery rate may be adversely affected (due to the time taken to metabolise the excess organic matter). Additionally, the time spent exceeding a given EQS may increase with successive occupations, as would the level of enrichment at the start of each new occupation, ultimately leading to the potential for "souring" of the site (Figure 7-11-B). In such a situation, either the organic flux (i.e. farming intensity) would need to be reduced, or the fallow period would need to be increased to achieve a sustainable cycle. Therefore, the influence that time spent in an 'accumulative' state has on recovery trajectories may be critical to the sustainability of fallowing strategies.

As such, when management goals are set around a maximum EQS, farm reintroductions should ideally gradually increase production (within practical constraints); allowing time for the benthos to adapt and deal with the additional flux of organic material, and then be maintained at a level that avoids the collapse of the macrofauna population. The sediment's ability to cope with organic inputs from fish farming, and hence the duration of the recovery period for a given location, is contingent on two key factors: the organic load in each farming cycle and the extent to which the sediment community is allowed to recover. Understanding the influence of each of these on sediment processes is important for sustainable long-term management of farming operations. If the overall recovery in any cycle is reduced then there is potential for the assimilative capacity of the sediments to be detrimentally affected, and a degenerative, and shortening, recovery cycle will likely ensue. Similarly, if the level of organic enrichment increases either in quantity or rate of input then the assimilative capacity of the sediments may be adversely affected. Under either of these situations management intervention may be required to bring sediment recovery back into line with the operational timeline.

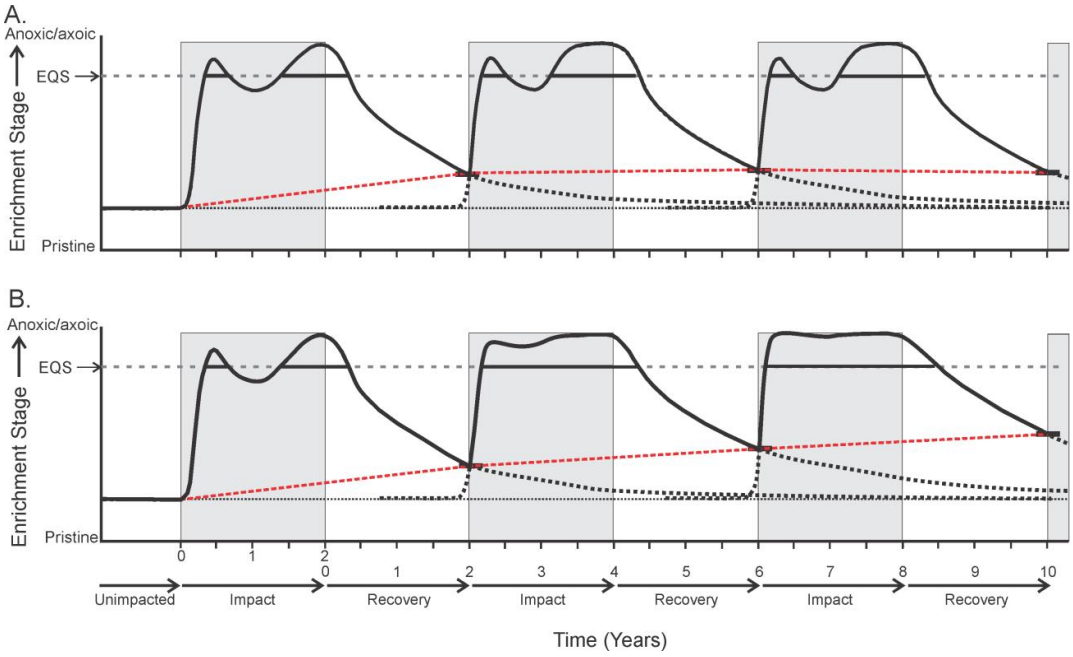


Figure 7-11: Theoretical recovery – re-impact profiles for a successive two year fallowing strategy. **A.** Semi-stable cycle - assumes the impact-recovery profile (and associated biological and chemical responses) is unaffected by residual effects at the point of farm reintroduction. **B.** Potential degenerative cycle - starts with the same recovery pathway, but assumes that the subsequent pathways are affected by the presence of a residual population of opportunistic taxa (and hence able to proliferate more rapidly) and that the recovery trajectory is negatively affected by more time spent in an accumulative state.

7.6 Acknowledgements

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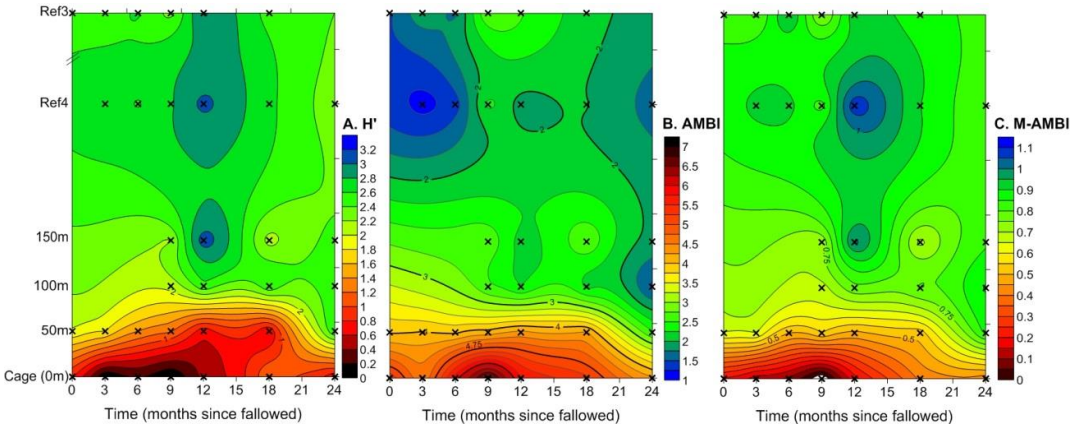
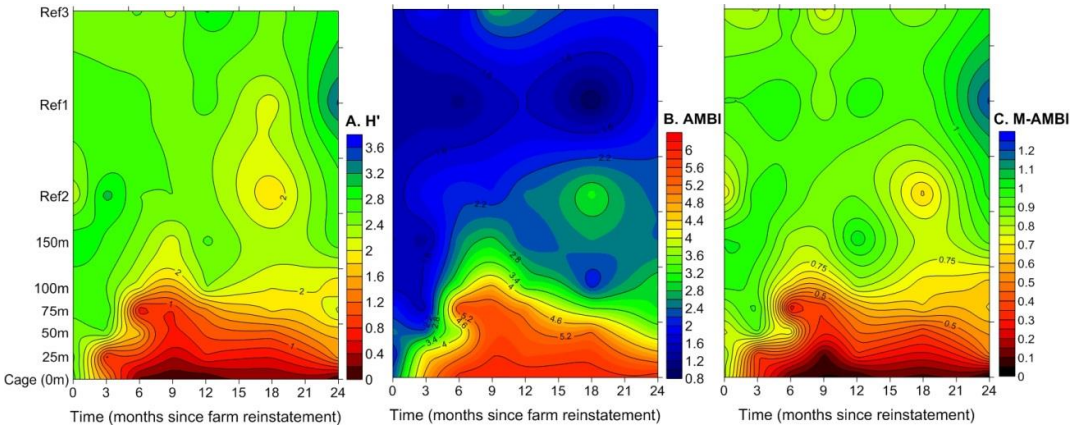
7.7 Appendix



Appendix 7-A: Changes in H', AMBI and M-AMBI with space and time during recovery at WAI. Time is given in months relative to when the cages were reinstated (M0) and space is represented as the square of distance (m) from the cages.



Appendix 7-B: Changes in H', AMBI and M-AMBI with space and time in response to farm reinstatement at FOR. Time is given in months relative to when the cages were reinstated (M0) and space is represented as the square of distance (m) from the cages.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 Quantifying benthic enrichment and the application of biotic indices

Prior to the commencement of the research described in this thesis, environmental monitoring of salmon farms was invariably conducted using a basic suite of indicators, which often differed among countries, regions and individuals. Assessments of farm compliance were, in many instances, reliant upon a subjective evaluation and a narrative description of overall benthic condition. Some countries have focussed on particular variables in an attempt to better quantify effects. For example Canadian scientists have been strong advocates of using total free sulphides (TFS) and redox as the primary indicators of benthic enrichment, on the basis that sediment chemistry drives the ecological response (Wildish et al. 2004, Hargrave et al. 2008, Hargrave 2010). But uncertainties surrounding the relationships with biological condition (Macleod et al. 2006), raised important questions about versatility and international applicability. Numerous biotic indices have been developed over the past 10 to 15 years, predominantly for the northern hemisphere (Pinto et al. 2009), with the purpose of evaluating benthic quality status, based on invertebrate ecology (or the biological response) but their implementation for salmon farm monitoring, in the Southern Hemisphere at least, was extremely limited. One of the primary aims of this thesis, was to develop a robust approach for accurately discerning levels of benthic enrichment, and hence involved testing the validity of these and other indicators.

Central to this process was the development of a common, unifying framework that could be used to compare and contrast both biotic and abiotic (physico-chemical) variables. This was achieved early in the research timeline using a process of average best professional judgement (Teixeira et al. 2010), as outlined in Chapter 2. The output was a bounded continuous variable, called Enrichment Stage (ES) that encapsulates all conceivable enrichment levels and places them on a scale from one (being pristine/ natural) to seven (being azoic / anoxic). This scale, conceptually represented in Figure 8-1, is to a large extent, anchored

around the point of peak abundance (ES = 5). This feature is essential to the applicability of the ES scale, as the proliferation of opportunistic taxa that drives the peak in total abundance is a key feature of the ecological succession gradient (Pearson & Rosenberg 1978, Glémarec & Hily 1981), underpins most biotic indices (e.g. Borja et al. 2000), and is a condition commonly associated with strong point-source discharges such as salmon farms (e.g. Buschmann et al. 2006). At the conclusion of this study, the ES framework had proven useful for a number of unforeseen purposes; most notably, it has provided a meaningful conceptual scale (represented in Figure 8-1) to which 'non-scientists' could relate, and has facilitated debate about what levels of enrichment should be considered appropriate in legislative / resource management arenas.

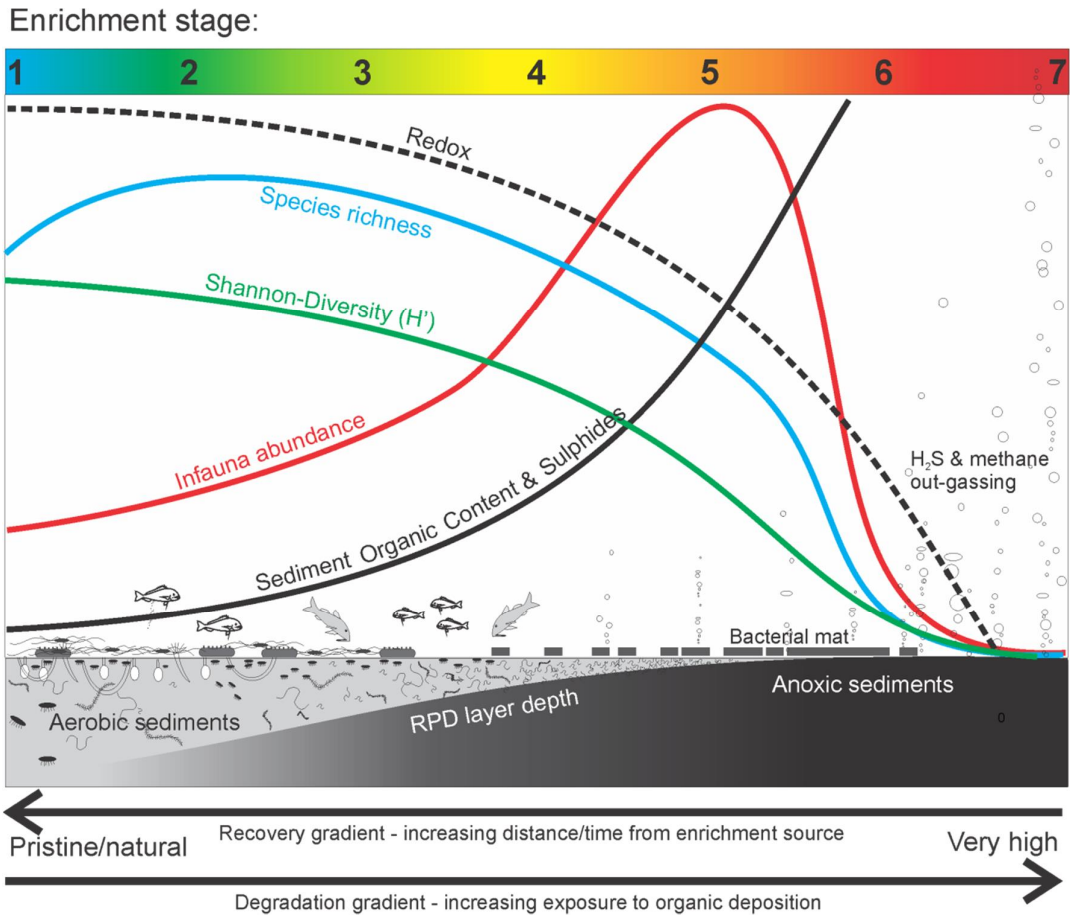


Figure 8-1. Conceptual diagram of Enrichment Stages (1 to 7)

In addition to development of ES as a new integrative variable, this thesis has also further evaluated the general applicability of existing biotic indices that are in common use internationally. One of the major factors limiting the broad applicability of these indices relates to the endemism of the species involved, and the extent of local knowledge surrounding the biology and pollution tolerances of macrofauna. For example, some of the potentially most useful biotic indices (e.g. BENTIX, MEDDOCC, AMBI and the related M-AMBI), require taxa to be assigned 'Eco-Group' (EG) classifications for use in the underlying calculations and formulae (e.g. Borja et al. 2000). Although viewing taxa in this way is not new to science (Glémarec & Hily 1981), the process of classifying the taxa can be highly subjective and usually requires consensus among experts (Borja & Muxika 2005), which can be a protracted process. The process of average best professional judgement adopted in Chapter 2 assigns an overall enrichment stage for a broad cross-section of samples, and therefore it is a relatively easy task to plot the abundance of individual taxa against this ES scale, and by fitting quantile regression splines, it is in turn possible to numerically determine enrichment tolerances. This regression method enabled the enrichment tolerances of 34 important indicator taxa to be quantitatively determined, and importantly, facilitated the assignment of EG classifications for 10 additional key taxa, for which our ecological understanding was previously limited. An analogous process was undertaken in Chapter 3 to allocate sensitivity/ tolerance scores used in the calculation of the BQI (ES500.05 for Benthic Quality Index based on Hurlbert 1971, Rosenberg et al. 2004) for 743 taxa by utilising a large existing macrofauna database. The outcome being that the BQI and other EG based indices (e.g. BENTIX, MEDDOCC, AMBI, M-AMBI) can now be reliably calculated for this region, and the systematic approach developed here is applicable to other locations and other forms of disturbance.

As noted in the preceding paragraph, assigning EG's to taxa has historically been undertaken by obtaining consensus among experts, which is important as it allows comparison of the results between regions (Borja & Muxika 2005). While this is valid in essence, the approach is premised upon a single species responding to enrichment in the same manner regardless of environment / region. The findings in Chapter 2 showed good agreement between the assignments resulting from the regression approach and the existing AMBI classifications (giving validity to the approach) for most of the shared taxa, however, there were also some significant differences observed. Part of this problem was attributed to taxonomic resolution; i.e. where higher level taxonomic groups are used there is significant scope for lower taxonomic-level specificity. However, some of the differences were at the

species level (i.e. the polychaetes *Cossura consimilis* and *Armandia maculata*), which implies that regionally-relevant classifications are needed. This is where the expert consensus approach fails, as different experts may have justifiably different experiences with the same species, and incorporating those experiences into a single EG is fraught with conflict. Therefore, it is apparent that any regional investigations into taxa-specific enrichment tolerances should provide improved performance of, and confidence in, the associated biotic indices. Given the common-place use of benthic invertebrates to assess impacts, this level of information should be viewed as fundamental to the effective management of marine ecosystems, rather than a luxury. However, there will inevitably be a point in the future where the effort required to obtain species-level biological information outweighs any potential benefits in terms of index performance; for example where rarer, numerically unimportant taxa are concerned. It is difficult to discern how far away this point is, as there is little known about the scale over which endemism can be important (it may be smaller than presently perceived), and the increasing use of molecular-based tools means that taxonomic resolution is a rapidly evolving concept (e.g. Huys et al. 2012, Martinez et al. 2013).

Despite the potential for future gains through obtaining a better understanding of macrofauna ecology, many existing biotic indices can be readily applied, and were able to be evaluated, along with other commonly utilised environmental indicators, under a variety of conditions in the Marlborough Sounds, New Zealand (Chapter 3). In total, 15 different metrics capable of indicating enrichment were contrasted using the enrichment stage framework, ranging from simple community statistics (e.g. total abundance) and sediment chemistry variables, to more complex biotic indices. This comparison led to two fundamental conclusions; i) several biotic indices performed well and were sensitive to changes lower on the enrichment scale (ES 2 to 3) - especially in comparison geochemical variables (redox and TFS), and ii) that most biotic indices do not perform well at very high levels of enrichment, i.e. beyond the peak in opportunistic species (PO, ES > 5). The second finding is significant because highly enriched conditions (i.e. ES5 – 7) often occur beneath salmon farms, and environmental compliance thresholds for locations at, or close to, the cages may require the ability to discriminate within this range. This 'ES6 conundrum' arises under quite specific circumstances - when diversity (or taxa richness) is very low, and abundance is diminishing substantively (post-peak) but the community remains dominated by the same one or two opportunistic taxa that occurred at ES5. This problem is more pronounced at low flow sites, where there is greater propensity for organic accumulation and sediment anoxia. At lower

levels of enrichment, the value of biotic indices over traditional geochemical measures is clearly evident, such as where it is necessary to detect the outermost spatial extent of impacts. To ensure that the full enrichment spectrum is reliably characterised, the solution proposed in Chapter 3 is to use a combination of variables that reflect complementary aspects of benthic condition, and as such, will work in combination to provide added power with regard to the detection of any enrichment gradient.

8.2 Which suite of variables best characterise enrichment?

Throughout these studies, reference has consistently been made to %OM, sulphides (and /or redox), species richness (S), total abundance (N), two or more biotic indices (i.e. AMBI and BQI) and ES as the key measures for determination of enrichment condition. These are viewed as the optimal suite of variables, as they provide measures of recent organic (carbon) loading, the oxic state of the sediments, and the time-integrated biological state with reference to peak abundance. This suite of variables can then be integrated to give an overall assessment of enrichment stage, as was demonstrated with the use of the ES variable throughout this thesis. ES puts the results on to a standard scale, thus enabling both scientists and non-scientist to relate to the condition assessment. As outlined above, there are recognised problems with relying on these variables in isolation, with the exception of ES, which integrates all available variables.

There is always pressure on regulators and scientists to reduce costs of compliance monitoring by developing new cost-effective methods, and / or rationalising expensive variables (e.g. macrofauna) in favour of cheaper indicator variables such as redox potential. An important over-arching conclusion from Chapters 2, 3 and 4 was that assessment of macrofaunal community composition is still the most reliable means of identifying enrichment effects, and is especially important at high flow sites where the response of %OM can be negligible and the relationships between geochemical and biological variables is more complicated. Unfortunately, macrofaunal composition is also the most labour intensive and expensive variable to assess. It may be justifiable in some instances to rely on a subset of indicator variables, however, this is only appropriate once the relationships between variables and, in particular ES, are understood for the site in question. From the variables that were used in this work, a suggested order of preference would be as follows: macrofauna count data

> TFS > redox > %OM. Although most expensive, an advantage to obtaining macrofauna count data is that it allows multiple biological indicators to be derived, which are individually relatively robust and can be combined to produce an overall biological assessment of enrichment stage.

Irrespective of the pros and cons of different variables, reliance on either biological indicators or sediment chemistry alone runs the risk of misinterpreting the overall benthic condition. For example, some of the inconsistencies that were observed between physico-chemical variables (%OM, redox, TFS) and the biology are likely due to both temporal processes and spatial dynamics. High %OM and TFS in combination with moderate diversity may be an indication that diversity is due to deteriorate. Conversely, a highly impacted macrofauna in the presence of near-natural sediment physico-chemistry may indicate a recovering state, as chemical remediation tends to be more rapid than biological remediation (Chapters 6 and 7). Or, in the case of a high flow site, the dispersive properties promote flushing and prevent organic accumulation and buffer against alterations to the sediment chemistry. Experience from these sites indicates that, given sufficient organic flux, the sediment chemistry can eventually become significantly altered – and this may occur relatively abruptly. Anticipating such biogeochemical changes would be very difficult in the absence of the more sensitive biological information, and hence both should be routinely monitored.

As a final note, it is worth reiterating that the methods used in these studies for quantifying TFS differed slight from those proposed by Wildish et al. (2004) in that a deeper profile was sampled (discussed in detail in Chapter 4). This may result in increased TFS values relative to those published elsewhere, and any future comparisons with this study should consider this influence.

8.3 Benthic enrichment in contrasting flow regimes

Another important theme throughout this study was the way that enrichment manifested at high flow sites compared to low flow sites. Of particular interest, was the observation that the relationships between biological and chemical variables proved to be characteristically different, with varying current speeds, and these differences were poorly described in the literature. This is a current and pertinent issue because there is an increasing

tendency (both nationally and internationally) to undertake finfish aquaculture at more exposed sites with stronger current speeds, and therefore, greater dispersive properties and oxygen delivery rates. This appeals to aquaculturalists for reasons of fish health and production (Kutty & Saunders 1973, Johansson et al. 2007) and greater environmental resilience (Frid & Mercer 1989, Findlay & Watling 1997, Borja et al. 2009a), and therefore it is assumed that the result will be greater farm capacity and sustainability. The analysis outlined in Chapter 4 showed that, contrary to popular assumptions, the macrofauna and geochemical variables at these high flow sites can become significantly impacted in what might appear to be the absence of organic accumulation, which has major implications for future industry development. The high flow sites examined in this study were able to support extreme abundances of opportunistic taxa, while simultaneously maintaining moderate species richness. The highest average total abundances recorded, in the order of 1.5 million m^{-2}, were also the highest known values reported in the literature to date. These extreme abundances of nematodes and unusually large capitellids were responsible for some significant deviations from the classical Pearson Rosenberg model (PRM), which was most evident in the biomass curve. The traditional PRM indicates a biomass peak at the early stages of enrichment in accordance with the peak in taxa richness (and the presence of more large-bodied animals); however, biomass at the high flow sites (and to a lesser extent, the low flow sites) clearly peaked in accordance with the peak of opportunists (PO), which occurs later in the enrichment gradient (or ES≈5).

Distinct flow-specific differences were also identified in the performance of the various environmental indicators (Chapter 3). None of the 15 metrics assessed were able to consistently discriminate over the full enrichment gradient for both flow environments. Number of taxa (S) and %OM were particularly poor indicators of environmental impact at high flow sites. S tended to remain high despite deteriorating geochemical conditions up until moderate to high levels of enrichment (ES≈4), at which point it declined abruptly. The most versatile indicators with regard to the contrasting flow environments were BQI > M-AMBI > AMBI > Log(N) > BENTIX; while AMBI best catered for different flow environments and the BQI was the least prone to erroneous responses under highly enriched conditions. Further analysis of the relationships between variables in Chapter 4, indicated that the decline in S coincided with the transition from oxic to hypoxic conditions according to TFS (1500 µM, Hargrave et al. 2008), suggesting that this may be a useful geochemical threshold for the avoidance of substantive declines in species richness at high-flow sites. The fact that %OM performed

poorly as an indicator of enrichment at high flow sites was most likely due to the strong currents that promote resuspension and prevents settlement and accumulation of organic particulates (Chapter 5 & Cromey et al. 2002b). Consequently, the results of Chapter 5 support the conclusion made in Chapter 4, that the greater resilience to organic waste at well-flushed sites was a function of both biological and physical processes. Collectively, these findings have important implications for the local benthic assimilation capacity, especially when considering the generally larger affected area (or 'footprint') associated with high flow sites that were identified in Chapter 5.

8.4 Predicting benthic enrichment and the potential for non-local effects

As a product of increased biodeposition (of fish feed and faeces), the effects of benthic enrichment that have been discussed to date should be predictable with the use of depositional models. One model in particular (DEPOMOD, Cromey et al. 2000) has been developed specifically for this purpose, but prior to commencing this research, remained largely untested in Southern Hemisphere systems. Confident application of such models requires local validation, and in this case, the hydrodynamic properties associated with two of the study sites provided a relatively novel testing environment due to their well flushed and highly dispersive nature. During some preliminary model runs, it was apparent that when the resuspension module is engaged (a feature used to predict the secondary entrainment and advection of particles post-settlement) the predicted net flux to the seabed was negligible, yet experience and real data at these sites indicated significant benthic impacts. A previous study had also identified that the resuspension module, and in particular the critical velocity threshold (*vr*, the velocity at which particles are resuspended), was an area that needed further validation (Chamberlain & Stucchi 2007). The emphasis of Chapter 5 was therefore to explore the applicability of DEPOMOD to high flow sites and extend the work of Chapters 2, 3 and 4 by establishing a link, or relationship, between predicted flux and observed effects. The ES variable once again proved useful, by providing a means of integrating and summarising the observed effects.

After extensive testing using known historical farm configurations it was concluded that localised benthic impacts may indeed be observed even where depositional models might

suggest otherwise, as significant benthic effects can occur in the perceived absence of organic 'accumulation' (Chapter 5). This finding was not entirely surprising as it is clearly consistent with findings of Chapters 3 and 4 regarding the characteristically different benthic effects found at dispersive sites. Three different velocity thresholds (9.5 (default), 12 and 15 cm s^{-1}) were tested to see whether it was simply a matter of the model over-predicting how much is being exported. However, the effect of raising the threshold to these levels was negligible, and raising it any higher was considered illogical. Instead, it was concluded that the model predictions using the default *vr* setting are reasonably accurate and that the observed impacts are truly occurring in the absence of any significant "net" organic accumulation. That being true, there remained a problem with predicting the effects at high flow sites; the predicted 'net' downward flux from the model was effectively zero and so cannot be used to give any indication as to either the magnitude or spatial extent of effects. This was overcome by effectively ignoring subsequent resuspension when generating the predicted flux, which is viewed here as the 'primary depositional footprint'. Using this approach the resemblance between the predicted primary footprint and the measured footprint was reasonably good, but clearly not perfect; the footprints at the high flow sites tended to be slightly larger and more diffuse than predicted, some of which is likely to be due to the resuspension processes.

The disparity between dispersive and non-dispersive sites was dealt with by deriving flow-specific regressions between predicted flux and observed enrichment stage. This revealed that approximately twice the amount of deposition flux is required to induce the same level of local benthic effects at dispersive sites compared to non-dispersive sites. While some of the additional flux at high flow sites may be accounted for by the greater benthic assimilation (Chapter 4), resuspension and advection processes clearly play an important role at high flow sites, which has implications for wider (non-local or far-field) ecosystem effects. Once advected, farm-derived organic particles will continue to decompose and be assimilated by a variety of organisms in the water column and / or situated on neighbouring structures. A portion will also settle out in neighbouring low flow areas where it may contribute to far-field benthic enrichment. These wider ecosystem effects are recognised but poorly understood and difficult to quantify (Sowles & Churchill 2004, Grant 2010). This is largely because the effects are likely to be relatively subtle and difficult to discern from that of other potential stressors such as sedimentation (from land-use practices, Chou et al. 2004), nonpoint source enrichment (Duda 1993), fishing (especially trawl and dredge fisheries, e.g. Jennings et al.

2001), global warming and ocean acidification and other large scale ecosystem anomalies (e.g. El Nino / La Nina).

There are sensitive approaches such as isotopic signatures (Sarà et al. 2006) or molecular methods (e.g. Maki et al. 2006) that are able to trace organic particulates in the far-field to determine the area of 'influence', but these are complex, relatively expensive, and the implications of the findings are limited. Although understanding the dispersion potential of wastes has some research value, the fact that conventional enrichment indicators are not useful at the same distances is significant, as it means the more sensitive 'forensic' results are not necessarily consistent with 'impact' or biological consequence as we know it today (Sarà et al. 2006). This raises the question, at what point does far-field enrichment become an important issue? The answer is a subjective one, but from an environmental perspective, it could be argued that ecological impacts would need to be realised for it to be considered problematic. Documented accounts of effects from far-field enrichment are scarce (probably for the reasons mentioned above), however there remains the underlying concern that effects may be cumulative, and by the time obvious effects are realised, there may already be large scale ecosystem changes. This is the rationale for adopting a precautionary approach, maintaining vigilance and continuing to explore potential areas of far-field enrichment and to develop methods for quantitatively measuring them.

This line of thinking also leads to the question – where is the ideal location for salmon farms, from the perspective of minimising environmental effects? Is it better to place a farm in a low flow environment where effects are extreme but highly localised, or in a high-flow environment where effects are less severe but more widespread? As is often the case, the answer will depend on site- and situation-specific circumstances. While physico-chemical impacts may be reduced in fast-flow environments, they are often associated with harder substrates (e.g. cobbles and reefs) and therefore benthic communities that are less well studied with respect to benthic enrichment and may be perceived to have relatively high ecological value. If these habitats contain sensitive or long-lived organisms, then the ability to recover may also be adversely affected (Hall-Spencer & Bamber 2007). In the Marlborough Sounds (New Zealand), for example, the shoreline adjacent to high-flow areas invariably include rocky outcrops which provide substrate for current-loving organisms such as hydroids, sponges, ascidians and macroalgae, which are often perceived to be sensitive to sedimentation. However, while there is a general paucity of studies describing enrichment

effects on reef-type assemblages, initial indications are that they are not readily impacted by enrichment (Dunmore & Keeley 2013). Additionally, if salmon farming ceased in fast-flow environments, or the cages were moved, recovery from impacts within the more conventional soft-sediment habitats may be considerably quicker due to the greater oxygen supply (Findlay & Watling 1997, Morrisey et al. 2000). Importantly, these arguments and comparisons only hold where farms are operated at similar production levels; if the intensity of feed use is increased at a high flow site such that the level of effects immediately beneath the Cages approaches those associated with a low flow farm, then the footprint would be larger, and the scope for far-field effects would be greater.

There are also potential disadvantages associated with low flow settings, the first relates to the possibility that the seabed is impacted to the point where it becomes "self-polluting". Highly anoxic sediments can deoxygenate overlying waters and produce methane and sulphide gasses, the latter being toxic to fish and indeed most animals (Hargrave et al. 2008). If systems are allowed to degenerate to this extent then the macrobenthos may become completely decimated, and the sites' ability to recover, should the farm be removed, will be further impaired relative to a high-flow setting. These disadvantages need to be offset against the recognised advantages of having a very localised footprint, and a macrobenthos that will likely be predisposed to dealing with additional organic matter (due to being situated in naturally depositional areas, e.g. Macleod et al. 2007). However, the findings of Chapters 4 and 5 further complicate this picture, suggesting that once impacted, the grater abundances and generally larger footprint typical of high flow sites may correspond to a greater ultimate assimilation capacity.

8.5 Benthic recovery and re-impact

The discussion thus far has dealt principally with static assessments of benthic condition. Chapters 6 and 7 extend this to consider spatial and temporal dynamics, particularly with regard to recovery pathways and related ecological succession. As noted earlier, recovery is a critical aspect of any environmental impact assessments (EIA) as it concerns the reversibility of the impacts and the associated temporal timeframes. Understanding recovery can also lead to conclusions about the potential for long-term cumulative effects (e.g. Lu & Wu 1998).

Chapter 6 examined recovery in space and time over an eight year period from a highly impacted state (ES6 – 7) at a low flow site, during which time recovery was assessed to have been ostensibly achieved after 5 years based on a weight-of-evidence approach. The only other study to consider recovery over a similarly long-term was conducted at a relatively unique ('worse case') site where chemical remediation was predicted to take 5 to 6 years and biological remediation much longer (Brooks et al. 2004). Others have identified substantially shorter timeframes for recovery (e.g. six months, Ritz et al. 1989), so clearly there are a number of factors that contribute to a system's ability to recover. Both Chapters 6 and 7 identified substantial recovery at the particular study areas in the first two years, by which time the Brooks et al. (2004) criteria for chemical remediation had been met and approximately half of the taxa had been reinstated. However, recovery was clearly not "complete" at the two year mark in either study, as evidenced by residual biological and geochemical impacts. These findings were generally consistent with the majority of studies that described substantive recovery in the first 6 to 24 months (Karakassis et al. 1999, Lin & Bailey-Brock 2008, Macleod et al. 2008, Villnas et al. 2011), but also concluded that recovery was not incomplete in the study timeframe (typically < 3 years). It was therefore significant that i) the dataset was sufficiently long that a potential end-point to recovery could be identified, and ii) the overall assessment is in the range of years, not months or decades, as this provides some additional confidence to evaluate potential effects in future fish farm developments.

The reasons underpinning the range of recovery estimates to date are also explored in Chapter 6, and were related to the degree of impact (or level of site use and intensity) at the point of fallowing, unusual environmental characteristics (e.g. Brooks et al. 2004, Macleod et al. 2007), and difficulties with defining a recovery end-point. Problems with defining the recovery endpoint were considered particularly pertinent and were attributed to: i) a high level of subjectivity regarding how recovery should be defined, ii) a dynamic environment, with changing reference conditions, and inherent instability in recovering sediments, iii) the relative change in the difference between impacted and reference sediments becomes increasingly small as the point of recovery approaches, with the result that there may be no obvious 'end-point', iv) different variables have different recovery responses, and v) no single test can encompass all of these aspects. Notably, Chapter 6 indicated the presence of a 'sliding background' (i.e. changing conditions at the reference station/s), and there was some evidence that alternate state theory (Beisner et al. 2003) may apply. However, the absence of an

accurate pre-impact baseline meant that the establishment of an alternate state could not be confirmed. Such considerations are likely to be applicable to many long-term studies of recovery and need to be considered in the analytical approaches.

Although the weight-of-evidence approach applied in Chapter 6 suggested recovery had occurred within the study timeframe, some differences and temporal instability in the composition of the macrofauna was still evident at the fallowed cage stations toward the end of the study, consistent with residual benthic impacts (Karakassis et al. 1999, Mendez & Linke-Gamenick 2001). There was also evidence to suggest that these temporal oscillations were partly due to farm-scale (10's of meters) spatial patchiness and migrations in sediments, as the peak in %OM and subsequently the biota shifted from directly under the fallowed cage stations to the gradient stations, 25 m to 50 m away. In Chapter 7 the concept of temporal and spatial instability was examined in more detail and a similar temporal shift was evident after 2 years, suggesting the observed oscillations were more than coincidental. The medium-term study (Chapter 7) also revealed oscillations between the oxic state of the sediments (via TFS) and biota, which may either be symptomatic of, or causal to, these spatial shifts in the sediments. Another interesting finding of the medium-term study was that similar oscillations were observed after the reintroduction of a farm in the presence of a consistently high load of additional organic flux. Thus abrupt change constitutes the disturbance that initiates environmental instability and this can be either the introduction or removal of an enrichment source.

The variables that were most consistent in their estimate of recovery were the more complex biotic indices, ES and multivariate methods that integrate across multiple variables. The use of more simplistic individual indicator variables such as total abundance, TFS or redox, runs the risk of identifying recovery prematurely. In contrast, using the more sensitive biotic indices in isolation are likely to indicate a longer recovery period, but would not necessarily acknowledge the fact that chemical remediation may have been complete for some time. Hence, there is clearly a need to predetermine the desired end-point criteria for recovery. By incorporating biotic and abiotic variables, the ES variable proved to be a reasonably stable and intuitive approach for evaluating the overall conditions during seabed remediation. However, this by no means eliminates the need to also examine the chemical and biological statuses individually. Six different approaches were evaluated in Chapter 6, one of which was a relatively novel test for 'parallelism' that was adapted from recovery assessments of the Exon

Valdez oil spill (Skalski et al. 2001). The overall conclusion was that no one method could be relied upon in isolation to accurately assess recovery.

This study has clearly shown that there is scope for developing better tools for evaluating recovery, not the least being more sophisticated statistical approaches to accommodate the many analytical challenges. In the meantime, the recommended methods involve a combination of visualisation of the plotted data with reference to estimates of background variability and tests for 'parallelism'. Future assessments of recovery would also benefit from some constructive discussion among scientists with a view to developing a universal definition for recovery, or at least an agreed range of definitions appropriate to specific circumstances.

8.6 Implications for fallowing and alternative mitigation practices

The parallel recovery and re-impact studies described in Chapter 7 suggest that fallowing strategies may be unsustainable unless multiple alternative sites are available and / or farming intensity, and therefore, the degree to which the seabed is impacted at the point of fallowing, is kept relatively low. However, the efficacy of fallowing strategies may be improved in the future with the implementation of alternative mitigation practices. Other sediment remediation techniques fall into three main groups: i) physical (e.g. waste collection and ploughing / harrowing O'Connor et al. 1993), ii) biological (and multi-trophic level aquaculture Vezzulli et al. 2004, bioaugmentation / biostimulation Kang et al. 2008) and iii) chemical (e.g. chemical injection, Hupfer & Hilt 2009, and activated carbon, Kupryianchyk et al. 2012). Of the many potential methods, a recent report by Eriksen et al. (2012) identified two as being most suitable for aquaculture and worthy of further investigation. These were:

1. Harrowing of sediments to increase oxygen penetration and carbon assimilation rates, and
2. "Soaker hoses" to increase oxygen penetration using oxygenated surface seawater pumped in to a network of hoses in or on the seabed.

These methods appear very much in the research stage; however, if they prove effective for accelerating the recovery process, then the practice of fallowing to manage benthic impacts could become generally more viable.

In Chapter 7 it was also identified that sediment condition might benefit from farms having the ability to progressively 'ramp up' farming intensity (or gradually introduce stock) to avoid 'overwhelming' the benthos. While introducing fish in small batches may not currently be feasible from a stock management and business perspective, there are other management practices that could be considered such as whether it may be possible to avoid introducing a fully functioning farm to a new site.

Interestingly, the potential for local and far-field enrichment effects is already being considered as part of integrated multi-trophic level aquaculture research, which considers the potentially beneficial utilisation of aquaculture waste products (e.g. Ren et al. 2012). Although current assessments of these practices suggest that they are not yet economic or practical, and / or limited in their effectiveness to significantly remediate local benthic effects (Bisset et al. 2009), the installation of artificial reefs has been shown to ameliorate benthic enrichment to some degree (Angel & Spanier 2002, Gao et al. 2008), and therefore may play a role in future management strategies. On the wider ecosystem-scale, it is conceivable that that some multi-species industries have evolved with a degree of co-dependency and mutual benefits in terms of productivity and nutrient management. For example, in the Marlborough Sounds, New Zealand there has been substantial concurrent development of extractive forms of bivalve aquaculture (predominantly mussels) and feed (and nutrient) additive finfish farms and it is likely that there is some trophic exchange and interaction that might benefit both operations.

8.7 Conclusions and recommendations

In conclusion, this research provides the basis for more confident evaluation of benthic enrichment effects (both from fish farming and other point sources). It has advanced our understanding of the fundamental ecology of several important macrofauna species and demonstrates how proven biotic indices and a suite of indicator variables can be combined and used collectively, in a quantitative (and largely non-subjective) manner, to evaluate overall enrichment stage. The work has also led to a generally expanded knowledge of the physico-chemical and biological processes associated with enrichment, particularly with regard to contrasting flow environments. In doing so it has highlighted a number of shortcomings and inconsistencies in the existing approaches to evaluating benthic enrichment effects.

Testing and validation of an established depositional model in a range of environments both highlighted some problems with the utilisation of the model in high flow areas due to the process of resuspension, but also provided a potential (interim) solution for predicting effects. Developing a better understanding of the role of resuspension for modelling purposes, and of the relationships between gross depositional flux and observed effects, was identified as a key area for further research. This work also extended the utility of the model by describing relationships between predicted depositional flux and observed ecological effects, and in doing so provided a means of gauging the likely capacity of sites in relation to any pre-determined benthic enrichment thresholds.

The third part of the research provided a detailed analysis of benthic recovery, and in doing so considered the fundamental but elusive concept of what constitutes recovery, and evaluated some relatively novel analytical methods for determining such an endpoint. Both the long- and medium-term studies of recovery incorporated a spatial dimension that helped explain the fluctuations that occur in time. The higher temporal sampling frequency of the medium-term study revealed a reciprocal, oscillating relationship between geochemical and biological variables that is likely to be part of the process responsible for the temporal variability that is associated with impacted / recovering benthos. By bringing together spatial scale, permanence (i.e. reversibility) and intensity concepts, the findings have contributed significantly to our ability to undertake Ecological Risk Assessments, which utilise these aspects (e.g. Forrest et al. 2009). It also brought together impact and recovery understanding to provide a more holistic evaluation of fish farm management approaches, with particular relevance for fallowing strategies.

Therefore, our overarching hypothesis was partially proven, and partially disproven - seabed enrichment can be accurately and quantitatively determined using biological and physico-chemical variables, both during degradation and recovery. But in order for the results to be robust the performance of individual indicators needs to be understood in the context of the given region and hydrodynamic conditions, and multiple indicators need to be combined to give an integrated result.

8.7.1 Recommendations for monitoring

This study has greatly enhanced our understanding of organic enrichment related processes both in general and in relation to finfish farming specifically. It has also highlighted some critical issues associated with farm location (geographic region and hydrodynamic condition), the implications of which are that "one size does not fit all" with respect to environmental monitoring and regulation. Hence, the emphasis on the importance of understanding local conditions in order to establish effective management practices, whether they be for regulatory or production purposes.

With respect to the original objective to develop or refine tools for the prediction, monitoring and management of enrichment effects associated with fish aquaculture, the following general recommendations can be made:

- In terms of a single assessment factor - benthic enrichment is most accurately quantified by assessing macrofaunal community structure and in particular Enrichment Stage (ES)
- Other sediment biogeochemical variables can help to clarify the picture. However, whilst sediment chemistry variables are cost effective, they can at times lack direct relevance to the ecological state and so are not to be recommended in isolation.
- The optimum suite of variables for accurate status assessment is: %OM, sulphides, species richness (S), total abundance (N), and two or more biotic indices (i.e. AMBI and BQI), but also including ES
- At dispersive sites %OM is of limited use – under these conditions it does not accumulate and is a relatively non-responsive indicator.
- Where natural conditions are well characterised, levels of acceptable change and BACI-type statistical tests (as described in Chapter 6) may be applied and may be more appropriate for detecting impacts.
- ES3 was a useful threshold level for classifying obvious / measurable effects in the local environment.
- Detection of subtle changes requires a multi-variable (weight of evidence) approach (e.g. multivariate analysis or ES) in order to reliably discern effects.

8.7.2 Recommendations for management

- To maintain ecological function and resilience of seabed sediment and biota, impacts should be managed so that ES5 (or point of peak abundance) is not exceeded. However, I would also note that this may be difficult to consistently achieve at low flow (accumulative) sites and as such should be considered an industry goal rather than a strict environmental quality standard.
- The following biogeochemical indicator levels are considered equivalent to an ES5 threshold and as such may be useful for general management:
    - Sulphide concentrations ~1500-2000 µM (this is also the point at which substantial reductions in species richness occur)
    - Up to a ~70% reduction in taxa richness
    - AMBI values >5 and M-AMBI values >0.2
    - Total abundance at the source (i.e. beneath the cages) should remain higher than at nearby, gradient stations.
- Depositional flux – ES relationships were developed that can be used to predict effects for new farms, and to optimise feed levels for new and existing sites. The key thresholds were:
    - Moderately / measurably enriched conditions (ES3) are induced with an additional depositional flux of ~0.4 kg m^{-2} yr^{-1} for non-dispersive sites and ~1 kg m^{-2} yr^{-1} for dispersive sites.
    - Highly enriched conditions (peak infauna abundance - ES5), are induced with an additional depositional flux of ~6 kg m^{-2} yr^{-1} and ~13 kg m^{-2} yr^{-1} for non-dispersive and dispersive sites, respectively.

8.8 Future directions

Throughout this research a number of areas have been identified where future efforts may be focussed in order to meaningfully advance the field of study. Many of these are discussed in the respective chapters and reiterated in the relevant section in the conclusions above, and as such will not be repeated here. However, there are some other potential extensions of this work that are worthy of mention. In particular, the ES framework that has

been used throughout this thesis is likely to have other applications beyond of the salmon farm context. Having a bounded continuous scale should prove useful for evaluating levels of enrichment from other sources, such as costal outfalls, river deltas and mussel farms that tend to be associated with lower levels of enrichment. This may require some further validation, particularly if applied to new areas with novel macrobenthic communities, however, the principle of the ES variable will provide a valuable context for considering the size and magnitude of any effects (providing a useful means for conceptualising the scales over which various levels of effects are acceptable / ecologically sustainable).

The ES framework will also provide a means of validating and testing new variables as they become available. There are a number of new and evolving technologies that have the potential to contribute to if not replace conventional benthic monitoring variables. Most notably, the techniques for DNA sequencing are rapidly evolving in capability and potential versatility. For example QPCR DNA sequencing of macrofauna is being used to monitor effects of the South Australia tuna farms (Loo et al. 2006), and next generation sequencing of macrofauna (Ranasinghe et al. 2012), foraminifera (Vidovic et al. 2009, Bouchet et al. 2012) and bacterial and archaeal ammonia oxidizers (Abell et al. 2010, Abell et al. 2011) show potential for discerning benthic enrichment gradients. But in order for these variables to be accepted as valid indicators the results will first need to be viewed in the context of the commonly accepted enrichment gradient to comprehend the relevance and to understand relationships to other aspects of ecology.

CHAPTER 9

GLOBAL REFERENCE LIST

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